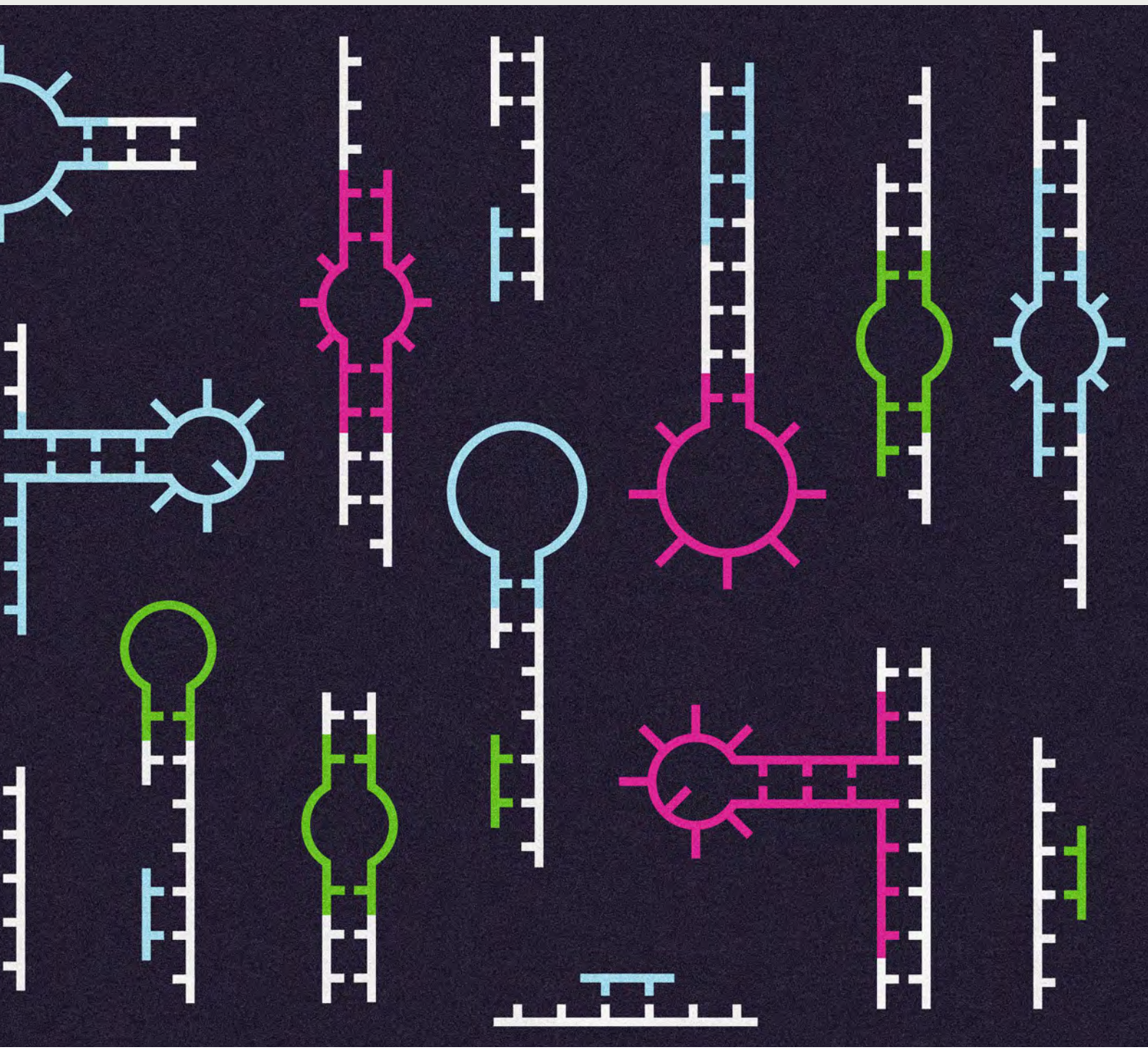
nature milestones

Antisense RNA



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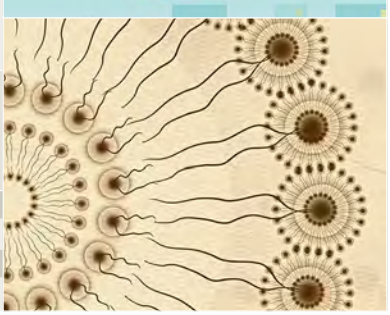
MILESTONES IN ANTISENSE RNA RESEARCH

1970s	Antisense cellular RNA and synthetic oligonucleotides inhibit mRNA translation (MILESTONE 1)
1990	RNA-mediated post-transcriptional gene silencing (PTGS) in petunia (MILESTONE 2)
1993	microRNAs emerge as novel post-transcriptional regulators of gene expression (MILESTONE 3)
1998	Double-stranded RNA mediates sequence-specific gene silencing in animals (MILESTONE 4)
	FDA approval of the first antisense oligonucleotide drug (MILESTONE 5)
1999	Small RNAs trigger different types of PTGS in plants (MILESTONE 6)
2000	Elucidation of the molecular mechanism of RNA interference (MILESTONE 7)
2001	First demonstration that small interfering RNAs trigger gene-specific silencing in mammals (MILESTONE 8)
	Discovery of PIWI-interacting RNAs (piRNAs) (MILESTONE 9)
2002	Advent of plasmid-based gene silencing and large-scale RNA interference screens (MILESTONE 10)
2005	Targeted gene silencing in vivo by antibody-mediated siRNA delivery (MILESTONE 11)
2012	Gene editing by CRISPR–Cas9, from bacteria to humans (MILESTONE 12)
2014	Chemical optimization enables targeted delivery of siRNA drugs to the liver (MILESTONE 13)
2016	FDA approval of an antisense oligonucleotide drug to treat spinal muscular atrophy (MILESTONE 14)
2018	First RNAi-based therapeutic agent approved by the FDA (MILESTONE 15)



'Waltz of the polypeptides' by Mara G. Haseltine, Cold Spring Harbor Laboratory, NY, USA. Credit: Ivanka Kamenova / Springer Nature Limited

MILESTONES

MILESTONE 1

First signs of antisense RNA activity

The first reports of antisense RNA activity in eukaryotes were published when the efforts to understand the mechanism of mRNA translation were well underway. At that time, cell-free in vitro translation systems were widely used to probe the function of different translation co-factors. These minimal systems, comprised of a cell extract and a template mRNA, proved instrumental in the initial characterisation of cellular RNAs that could control mRNA translation through antisense recognition of their targets.

Working in such a cell-free system, in 1975, Stuart Heywood and colleagues demonstrated that short ribonucleotide sequences purified from chicken muscle messenger ribonucleoprotein and polysome fractions could control translation of the mRNA encoding myosin. They called the RNAs 'translation control RNAs' (tcRNAs) and proposed that tcRNAs act by binding to their mRNA targets in a sequence-specific manner. In follow-up work a decade later, Heywood demonstrated that one of these tcRNAs, tcRNA102, recognises a sequence in the 5′ untranslated region of chicken myosin mRNA, albeit with imperfect homology.

In the years immediately following Heywood's original tcRNA discovery, several groups isolated similar small RNA species from different organisms and demonstrated that these short RNAs could modulate translation. Notably, in 1977, Severo Ochoa and colleagues purified two distinct short RNAs from a small crustacean, *Artemia salina*, and showed that these RNAs exerted activating and inhibitory effects on *A. salina* mRNAs. The activator RNA is complementary to the inhibitory RNA, and thus its stimulatory effect on translation was proposed to be due to sequestering the inhibitory RNA through base-pairing. The ideas put forward by Ochoa — that small regulatory RNAs exist as double stranded structures and are generated by endogenous RNase enzymes — have been revisited in subsequent decades, as the mechanisms for processing of small interfering RNAs and microRNAs were uncovered.

Whereas the above reports described the activity of cellular antisense RNAs, in 1977, Paterson et al. demonstrated that exogenous plasmid DNA fragments complementary to an mRNA could also inhibit its translation in vitro. A year later, Paul Zamecnik and Mary Stephenson reported the first synthetic DNA oligonucleotide delivered to cells, capable of inhibiting viral replication and oncogenic transformation caused by Rous sarcoma virus. The 35S RNA of this retrovirus had recently been found to contain a 20-nucleotide repeat sequence in its 5′ and 3′ ends. The authors synthesized 13-nucleotide-long DNA oligonucleotides complementary to part of the viral repeat sequence, which was hypothesized to be important for viral replication, and tested their effects on the growth of chicken embryonic fibroblasts infected with Rous sarcoma virus. Two synthetic oligonucleotide variants were tested: one with free 3′ and 5′ termini, and one with chemical modifications. Strikingly, addition of either oligonucleotide to the cell culture medium at the time of infection inhibited viral replication and oncogenic transformation of the cells. The chemically modified oligonucleotide performed better, likely because the modifications conferred resistance to nucleases in the culture medium.

The most likely target processes of the complementary oligonucleotide were the circularisation of provirus DNA or the translation of viral mRNA. Although the first possibility was not ruled out, in a companion paper published in the same issue of *Proceedings of the National Academy of Sciences*, Stephenson and Zamecnik demonstrated that the delivered oligo inhibits translation of viral mRNA through sequence-specific hybridisation.

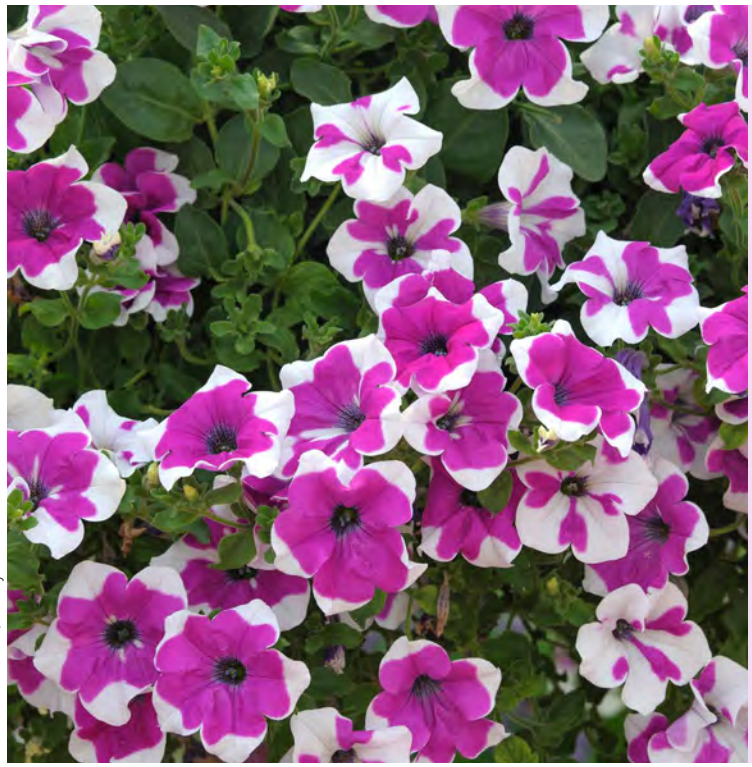
Our knowledge of the mechanisms by which endogenous RNAs regulate key processes in cell function and in development has since grown exponentially. Yet, these early experiments, using highly purified components, provided the first hints that cells produce small RNAs with the capacity to affect translation, and that the complementarity of these RNAs to their target is important for their function. The experiments by Zamecnik, Stephenson and others laid the foundation of the field of antisense RNA therapeutics and resulted in the founding of Hybridon, the first biotechnology company dedicated to developing synthetic oligonucleotides for therapeutic purposes.

Ivanka Kamenova, Associate Editor, *Nature Protocols*

MILESTONE STUDIES Bester, A. J. et al. Two classes of translational control RNA: their role in the regulation of protein synthesis. *Proc. Natl Acad. Sci. USA* **72**, 1523–1527 (1975) | Lee-Huang, S. et al. Eucaryotic oligonucleotides affecting mRNA translation. *Arch. Biochem. Biophys.* **180**, 276–287 (1977) | Paterson, B. M. et al. Structural gene identification and mapping by DNA–mRNA hybrid-arrested cell-free translation. *Proc. Natl Acad. Sci. USA* **74**, 4370–4374 (1977) | Zamecnik, P. C. et al. Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide. *Proc. Natl Acad. Sci. USA* **72**, 280–284 (1978) | Stephenson, M. L. et al. Inhibition of Rous sarcoma viral RNA translation by a specific oligodeoxyribonucleotide. *Proc. Natl Acad. Sci. USA* **75**, 285–288 (1978).
FURTHER READING Heywood, S. M. tcRNA as a naturally occurring antisense RNA in eukaryotes. *Nucleic Acids Res.* **14**, 6771–6772 (1986) | Zamecnik, P. C. From protein synthesis to genetic insertion. *Annu. Rev. Biochem.* **74**, 1–28 (2005) | Kresge, N. et al. The Discovery of tRNA by Paul C. Zamecnik. *J. Biol. Chem.* **280**, e37 (2005).

MILESTONE 2

Patterns of co-suppression in plants



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> …experiments on pigmentation in petunias did not simply have unexpected results, but results completely opposite to what had been anticipated, arguably marking the beginning of the field of RNA interference

It is often said that truly novel discoveries arise when experiments do not go as planned. This is certainly true of one of the earliest observations of an RNA-mediated gene silencing mechanism by Richard Jorgensen and colleagues at the DNA Plant Technology Corporation in Oakland, California. Their experiments on pigmentation in petunias did not simply have unexpected results, but results completely opposite to what had been anticipated, arguably marking the beginning of the field of RNA interference (RNAi).

During the 1980s, the ability to transform plant species using *Agrobacterium tumefaciens* had opened up the possibility of creating transgenic plants with traits desirable for agriculture, such as herbicide-resistant crops, by the introduction of genes of bacterial origin. Another goal was the creation of new varieties of ornamental plants by modifying their pigment synthesising pathways.

Jorgensen's group was working on the popular annual *Petunia hybrida*, attempting to deepen its colouration by increasing the number of copies of the gene encoding the enzyme chalcone synthase (*CHS*). CHS catalyses the condensation of one molecule of 4-coumaroyl-CoA and three molecules of malonyl-CoA to form naringenin chalcone, from which a wide variety of flavonoid pigments are synthesised.

Agrobacterium was used to introduce multiple copies of the *CHS* gene, under the control of a cauliflower mosaic virus 35S promoter, into three different petunia varieties. Not a single plant with deepened colouration resulted from these transformations. Instead, many of the transformed flowers were completely white, and others produced variegated patterns containing more white regions than the original plants.

These patterns were stable, with individual plants producing hundreds of blooms, all patterned in the same way. Very occasionally a plant would start producing differently patterned flowers or reverting to the parental form on a side branch. If cuttings were taken from the branches, the new individuals would have the same flower pattern as the branch from which they were cut.

Back-crossing the transformed plants to parental lines allowed the researchers to show that the altered pigmentation only occurred when the transgene was present and active. RNase protection assays using radiolabelled RNA probes antisense to the gene showed that messenger RNA levels for both endogenous and transgenic *CHS* in transgenic plants were reduced or essentially abolished.

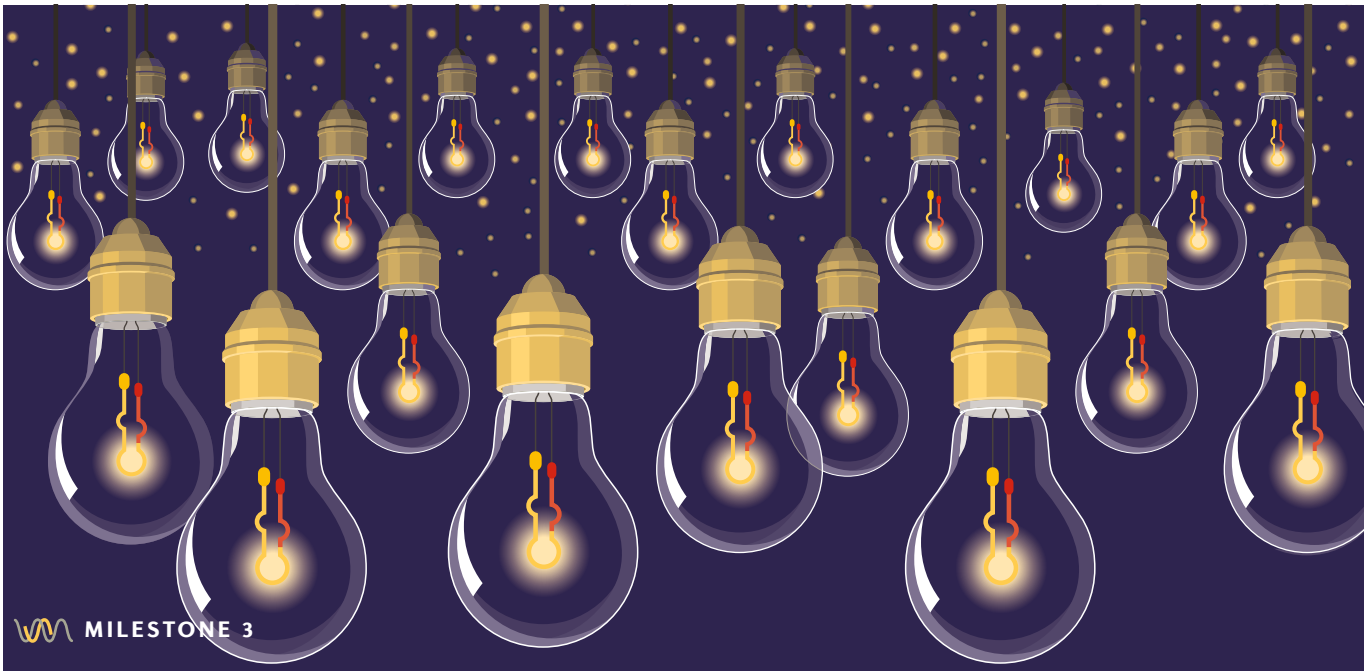
It would have been very easy for Jorgensen and his team to dismiss these results as a failed experiment, not least because a similar approach attempted by a group at the University of Amsterdam had not produced the dramatic phenotypes seen by the Californians. However, discussions between the two groups identified subtle differences in growing conditions. Increasing the light levels resulted in the Dutch petunias confirming Jorgensen's observations.

These experiments showed clearly that co-suppression of gene expression was dependent on high-copy-number transcription of a transgene homologous to a native gene, but was not affected by the proximity of those genes in the plant genome.

It would take almost a decade for the role of this form of RNAi as a defence against plant viruses to be identified, and the complexities of the underlying mechanisms are still a topic of research. In this respect, the 1990 paper was remarkably prescient in deducing from the "erratic and reversible nature of the transgene effect" that DNA methylation was involved.

Chris Surridge,
Chief Editor, *Nature Plants*

MILESTONE STUDY Napoli, C. et al. Introduction of a chalcone synthase gene into Petunia results in reversible co-suppression of homologous genes in trans. *Plant Cell* **2**, 279–289 (1990).
FURTHER READING van der Krol, A. R. et al. Flavonoid genes in petunia: addition of a limited number of gene copies may lead to a suppression of gene expression. *Plant Cell* **2**, 291–299 (1990).



MILESTONE 3

microRNAs emerge as potent post-transcriptional gene regulators

There is nothing more precise than a Swiss watch — besides the pattern of development of the nematode worm *Caenorhabditis elegans*, one of the most studied and useful animal models. The postembryonic development of *C. elegans* entails passage through four accurately coordinated larval stages (L1–L4) interspersed with moults. In the mid-1980s, many scientists were interested in genetic aberrations that could alter the precise timing of *C. elegans* development. Genes that, when manipulated, could delay or advance the nematode's cell cycle and developmental-stage progression were called heterochronic genes. As expected at the time, most of these genes encode proteins.

In 1993, Victor Ambros and colleagues demonstrated that down-regulation of the protein LIN-14 was crucial for the progression from the first larval stage (L1) to the second larval stage (L2). Loss-of-function mutations in *lin-14* cause *C. elegans* to skip a beat, starting development from L2. On the other hand, mutations in another gene, *lin-4*, halted developmental progression indefinitely at the L1 stage. Surprisingly, *lin-4* did not encode a protein; instead, it is transcribed into a small non-coding RNA with sequence complementarity to the 3′ untranslated region (3′ UTR) of *lin-14*. Lin-4 was the first microRNA to be discovered.

At the same time, Gary Ruvkun and colleagues showed that binding of lin-4 to the 3′ UTR is essential for LIN-14 downregulation. Both teams correctly hypothesised that lin-4 pairs through antisense complementarity to the 3′ UTR of *lin-14*, and forms an RNA duplex that leads to translational repression of *lin-14*. Although lin-4 binding did not affect the overall mRNA levels of *lin-14*, it decreased LIN-14 protein expression, subsequently causing progression from L1 to L2.

This novel mechanism of post-transcriptionally regulating gene expression was shown, in both articles, to be conserved in several worm species, but at the time it was mostly thought to be a nematode oddity. During the 1990s, a second microRNA regulating *C. elegans* development was identified and named let-7. In the case of let-7 mutant nematodes, larvae stopped just short of becoming adult worms. Lin-4 and let-7 were quite different from each other but, in 2000, Ruvkun and colleagues found homologues in the genomes of *Drosophila melanogaster* and *Homo sapiens*. Although humans have no heterochronic genes, fruit flies do, and the temporal expression profile of let-7 was shown to be conserved between worms and fruit flies.

> “… although lin-4 binding did not affect the overall mRNA levels of *lin-14*, it decreased LIN-14 protein expression…”

Since the discovery of lin-4 and let-7, many microRNAs have been identified. This family of small non-coding RNAs is involved in the regulation of diverse biological processes, and includes many potential therapeutic targets — not bad for what were originally thought to be mere worm time-keepers.

Anne Mirabella,
Senior Editor, *Nature Communications*

MILESTONE STUDIES Lee, R. C. et al. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell* **75**, 843–854 (1993) | Wightman, B. et al. Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in *C. elegans*. *Cell* **75**, 855–862 (1993).
FURTHER READING Pasquinelli, A. E. et al. Conservation of the sequence and temporal expression of *let-7* heterochronic regulatory RNA. *Nature* **408**, 86–89 (2000).

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MILESTONE 4

Exogenous dsRNA silences genes in *C. elegans*

In 1998, antisense RNA was known to regulate gene expression in cell lines, plants and worms. It was puzzling, however, that in the nematode *Caenorhabditis elegans*, injection of either sense or antisense RNA resulted in transcription interference that could be transmitted to offspring. Fire, Mello and colleagues aimed to uncover some of the mysteries behind what they coined 'RNA interference' (RNAi). They studied the twitching phenotype caused by a reduction in the expression of the gene *unc-22*; complete loss of *unc-22* expression results in more severe muscular defects and impaired motility.

To examine whether single-stranded RNA (ssRNA) or double-stranded RNA (dsRNA) contributed to the twitching effect, a 742-nucleotide ssRNA homologous to *unc-22* was purified and its ability to silence *unc-22* relative to the homologous dsRNA was compared. Whereas the ssRNA produced only incremental effects, co-injection of sense and antisense RNA, rather than consecutive injections, was highly effective in producing a twitching effect in the adult worm. This phenotype was heritable, although progeny were expected to maintain only a few RNA molecules per cell at the 500-cell stage, which is when *unc-22* expression begins.

That gene silencing was likely mediated by dsRNA was further inferred by the fact that injecting the worms with gel-purified dsRNA phenocopied silencing. No effect was observed after injection of control dsRNA that was either not related to or that targeted promoter or intronic regions of *unc-22*. Gene silencing effects could be reproduced by dsRNA, but not ssRNA, that was homologous to three other genes with well-characterized phenotypes. At this point, the authors deduced that dsRNA was involved, that the stoichiometry between the dsRNA and the endogenous target mRNA was not required to be 1:1, and that the response was specific to the targeted mRNA.

To visualize the silencing effect, the authors used *mex-3*, a transcript that is abundant in early embryos and can be easily detected using in situ hybridization. They found that the *mex-3* transcript was not detectable following injection of dsRNA derived from *mex-3*. Surprisingly, injection of purified *mex-3* antisense RNA did not significantly affect *mex-3* transcript levels. Another unexpected finding was that regardless of where in the worm dsRNA was injected, gene silencing was observed in the somatic tissue of the injected worm as well as in its progeny, suggesting the involvement of RNA transport.

> "… dsRNA… response was specific to the targeted mRNA"

Although the mechanism of RNAi remained unclear at the time, this seminal work was the first to show that strong gene silencing can be mediated by dsRNA. It laid the ground-work for a decade of studies that characterized the molecular mechanism underlying the RNAi pathway (MILESTONE 7) and showed that RNAi is a widespread endogenous phenomenon.

Faten Taki, Associate Editor, *Communications Biology*

MILESTONE STUDY Fire, A. et al. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**, 806–811 (1998).
FURTHER READING Nellen, W. & Lichtenstein, C. What makes an mRNA anti-sense-itive? *Trends Biochem. Sci.* **18**, 419–423 (1993) | Fire, A. et al. Production of antisense RNA leads to effective and specific inhibition of gene expression in *C. elegans* muscle. *Development* **113**, 503–514 (1991).

The 2006 Nobel prize in physiology or medicine is awarded to Andrew Fire and Craig Mello for their discovery of RNA interference — gene silencing by double-stranded RNA.

Credit: Editorial / Alamy Stock Photo

MILESTONE 5

First antisense drug is approved with fleeting success

> …the success of [this drug] provided proof-of-concept of the clinical promise of treatments based on antisense oligonucleotides…

Following the initial reports and characterization of gene silencing through endogenous and exogenous antisense RNAs, the first antisense oligonucleotide drug, fomivirsen, received regulatory approval from the United States Food and Drug Administration (FDA) in 1998. This agent was indicated for the treatment of cytomegalovirus (CMV) retinitis — a serious infection of the retina that can rapidly lead to blindness — in carriers of human immunodeficiency virus (HIV) exhibiting acquired immune deficiency syndrome (AIDS), who were intolerant of, or had contraindications to, other treatments or were insufficiently responsive to previous treatments. Fomivirsen was subsequently granted marketing authorisation by the European Medicines Agency (EMA; formerly the EMEA) for the same indication in 1999.

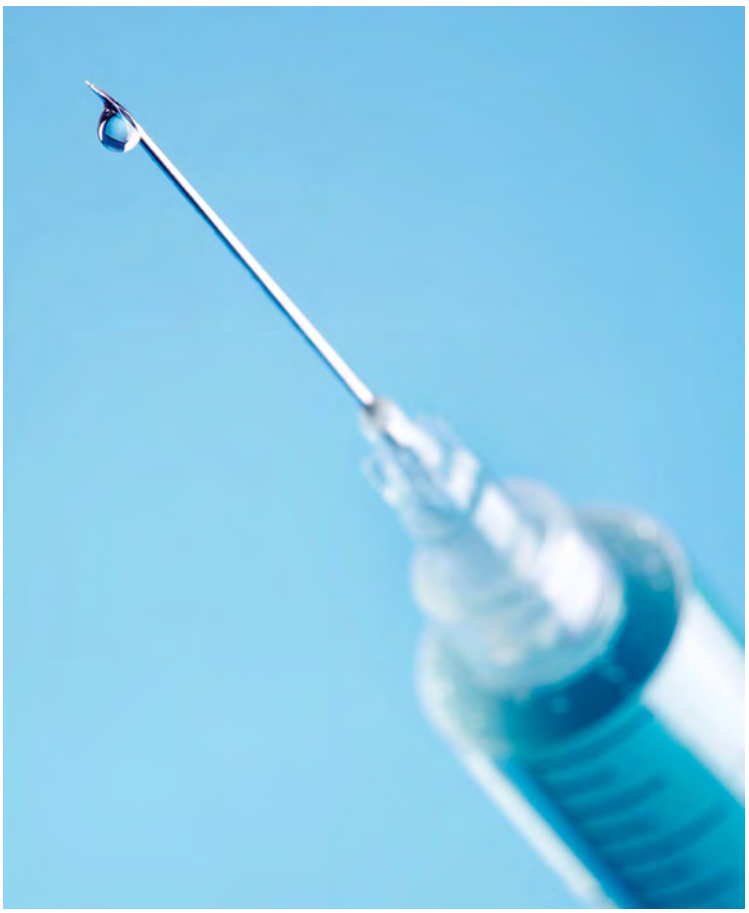
Fomivirsen is a synthetic 21-nucleotide phosphorothioate oligodeoxynucleotide designed to be complementary to a sequence in CMV mRNAs encoding the major immediate-early region 2 proteins, which are essential for CMV replication. In line with this antisense mechanism of action, early preclinical characterization revealed that fomivirsen potently and selectively disrupted CMV replication in a dose-dependent manner in vitro, providing the first indication of its efficacy.

Regulatory approval was largely based on three prospective randomized controlled trials (RCTs) led by the Vitravene Study Group, which demonstrated the efficacy of fomivirsen for the treatment of CMV retinitis in individuals with AIDS. In a pivotal phase III RCT, patients with newly diagnosed CMV retinitis were randomly allocated to immediate intravitreal fomivirsen treatment or treatment deferral until progression. Median time to progression was 71 days in the immediate treatment group, versus 13 days in the deferred treatment group. Progression after treatment cessation occurred in 44% of patients receiving immediate treatment, versus 70% of patients in the deferred treatment group. Two additional RCTs demonstrated the comparable efficacy of an intensive and less-intensive regimen of intravitreal fomivirsen for the treatment of CMV retinitis that had not been controlled by other drugs.

However, despite the initial enthusiasm and unmet clinical need in the late 1990s, the success of fomivirsen was ultimately fleeting. The drug was withdrawn by the FDA in 2001, owing to the success of highly active antiretroviral therapy in reducing the incidence of opportunistic infections in individuals with HIV in the early 2000s, which undermined demand for fomivirsen. The EMA followed suit in 2002, when the manufacturer (Novartis) voluntarily withdrew the drug from the market due to low demand.

Nevertheless, the success of fomivirsen provided proof-of-concept of the clinical promise of treatments based on antisense oligonucleotides, which was undoubtedly valuable for the next wave of antisense drug approvals, beginning in 2013 with the FDA approval of mipomersen (an antisense oligonucleotide inhibitor of apolipoprotein B) for the treatment of homozygous familial hypercholesterolemia.

Conor A. Bradley, Senior Editor, Nature Reviews Cross-Journal Team



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FURTHER READING *Drug Approval Package: Vitravene* (US FDA, 2002); https://go.nature.com/2kfsM3N | *Vitravene* (EMA, 2002); https://go.nature.com/2kJcOze | Vitravene Study Group. A randomized controlled clinical trial of intravitreous fomivirsen for treatment of newly diagnosed peripheral cytomegalovirus retinitis in patients with AIDS. *Am. J. Ophthalmol.* **133**, 467–74 (2002) | Vitravene Study Group. Randomized dose-comparison studies of intravitreous fomivirsen for treatment of cytomegalovirus retinitis that has reactivated or is persistently active despite other therapies in patients with AIDS. *Am. J. Ophthalmol.* **133**, 475–83 (2002).

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MILESTONE 6

Small RNAs trigger silencing in plants

By the late 1990s, many instances of post-transcriptional gene silencing (PTGS) in plants had been reported. The phenomenon was known to be a defence mechanism that could target endogenous sequences (for example, transposable elements), transgenes or viral RNAs. When Fire and Mello described RNA interference in *Caenorhabditis elegans* (MILESTONE 4), it was immediately obvious that PTGS is the plant equivalent of RNA interference.

There are different types of PTGS in plants. Originally reported in petunia, PTGS induced by a transgene with homology to an endogenous gene leads to 'co-suppression' of both the transgene and the endogenous gene (MILESTONE 2). Transgene-induced PTGS, however, can also occur when the transgene has no homology to endogenous sequences, causing silencing of only the transgene. Systemic PTGS is first triggered locally, then spreads to other tissues. Finally, PTGS can also be mediated by viral RNAs. In all these types of PTGS, cellular accumulation of RNAs with homology to either a transgene or to viral sequences is suppressed, suggesting that the silencing mechanism requires sequence specificity, but the silencing trigger remained elusive.

In 1992, Richard Jorgensen proposed that ectopic pairing of homologous DNA might initiate PTGS. This model was supported by subsequent studies, and antisense RNAs were suggested to initiate silencing by forming a duplex with the targeted mRNA, thereby facilitating its degradation or translation inhibition. However, no antisense RNA molecules with sizes comparable to typical mRNAs had been detected.

In 1999, Hamilton and Baulcombe hypothesized that the antisense RNA trigger might be too short for detection by standard RNA analysis techniques. Therefore, they designed experiments to specifically hunt for short antisense RNAs in the different types of PTGS in plants. Strikingly, they detected 25-nucleotide (nt) antisense and sense RNAs in two tomato lines that exhibited transgene co-suppression of the *ACO* gene.

> "…these studies established low-molecular-weight RNAs as the trigger of PTGS and RNA interference, and showed that pairing of the antisense strand with the target mRNA mediates mRNA degradation"

These RNAs were absent in control plants. In tobacco plants carrying a *GUS* transgene without sequence homology to endogenous sequences, 25-nt antisense RNAs were also specifically detected in plants that exhibited PTGS. Furthermore, by inoculating a single leaf of a GFP-transgenic *Nicotiana benthamiana* plant with *Agrobacterium tumefaciens* containing GFP sequences in a T-DNA vector, the researchers observed systemic silencing of GFP and detected 25-nt GFP antisense RNAs in all affected tissues. Finally, in *N. benthamiana* infected by potato virus X, 25-nt antisense RNAs were also found only in infected leaves. Thus, 25-nt antisense RNAs were present in all types of PTGS examined.

As 25-nt RNAs are long enough to confer sequence specificity, but are short enough for transport through plasmodesmata, Hamilton and Baulcombe proposed that these small RNAs are the likely triggers of PTGS. However, they did not know at the time how the small RNAs were generated. The antisense character of the 25-nt RNA species and its presence in RNA-virus-infected cells indicated that these short RNAs were generated from an RNA template rather than a DNA template. Only a little while later, scientists working in animal systems reported that long double-stranded RNA (dsRNA) is processed into 21–23-nt dsRNA fragments that guide mRNA cleavage in vitro (MILESTONE 7). In vivo evidence followed, demonstrating that long dsRNAs are cleaved to about 25 nt and that the antisense strand triggers mRNA degradation by pairing to target mRNAs. Together, these studies established low-molecular-weight RNAs as the trigger of PTGS and RNA interference, and showed that pairing of the antisense strand with the target mRNA mediates mRNA degradation, but the molecular machinery involved remained to be discovered.

Jun Lyu,
Senior Editor, *Nature Plants*

MILESTONE STUDY Hamilton, A. J. & Baulcombe, D. C. A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science* **286**, 950–952 (1999).
FURTHER READING Jorgensen, R. Silencing of plant genes by homologous transgenes. *Agbiotech News Info* **4**, 265–273 (1992).

MILESTONE 7

Mechanism of RNA interference discovered

Following the landmark discovery that exogenously provided double-stranded RNA (dsRNA) reduces the expression of homologous mRNAs in *Caenorhabditis elegans*, and the suggestion that such RNA interference (RNAi) may have a catalytic component (MILESTONE 4), gene silencing triggered by dsRNA was observed in many species including *Drosophila melanogaster*, trypanosomes, fungi and plants. However, the mechanism of unwinding energetically-stable dsRNA to promote the search for complementary targets remained unknown.

In 2000, two papers reported the elucidation of the mechanisms underlying RNAi using biochemical approaches. These papers suggested that the key to RNAi is the conversion of dsRNA into small RNAs, which then guide specific cleavage of complementary targets.

Work from the Hannon laboratory showed that incubation of synthetic mRNAs with extracts of *D. melanogaster* S2 cells transfected with dsRNA recapitulates RNAi in vitro. The activity responsible for the sequence-specific mRNA degradation was termed RNA-induced silencing complex (RISC).

> “… the RNAi-active fraction was shown to contain ~25 nt-long small RNAs that were homologous to target mRNAs”

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Pre-treatment of extracts with micrococcal nuclease abolished RISC activity, whereas DNase I had no effect, suggesting that RISC contains RNA components. By purifying RISC activity using chromatography, the RNAi-active fraction was shown to contain ~25 nt-long small RNAs that were homologous to target mRNAs. This was in line with the presence of ~25 nt small RNAs during post-transcriptional gene silencing in plants (MILESTONE 6).

Zamore, Tuschl and colleagues provided more direct evidence of the active role of small RNAs in RNAi using *D. melanogaster* embryo lysates and radiolabelled RNAs. Both strands of long dsRNAs were processed into 21–23 nt small RNAs. Interestingly, the cleavage products of the target mRNAs were produced at 21–23 nt intervals, the same interval as during dsRNA processing. These results suggested that small RNAs guide the cleavage of target mRNAs.

Shortly thereafter, several laboratories were able to identify enzymes and cofactors in the RNAi pathway using genetic and biochemical methods. These efforts revealed that dsRNAs are cleaved by the RNase III enzyme Dicer to generate small RNAs, which were termed small interfering RNAs (siRNAs). RISC comprises an Argonaute protein loaded with siRNA, which cleaves complementary mRNAs.

The discovery of the mechanism of RNAi revolutionised experimental gene regulation. siRNAs targeting complementary mRNAs can be easily designed and rapidly synthesized. Of clinical relevance is the targeting of multiple mRNA molecules by the same siRNA molecule in consecutive rounds of base-pairing, compared with drugs that remain bound to their target molecule. Thus, the discovery of RNAi mechanisms has laid the groundwork for the development of RNAi-based therapeutics.

Minju Ha,
Associate Editor, *Nature Communications*

MILESTONE STUDIES Hammond, S. et al. An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature* **404**, 293–296 (2000) | Zamore, P. D. et al. RNAi: Double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. *Cell* **101**, 25–33 (2000).

MILESTONE 8

Small interfering RNAs silence genes in mammals

The discovery that exogenous double-stranded RNA (dsRNA) could silence specific genes in *Caenorhabditis elegans* (MILESTONE 4) was closely followed by the observation that, in *Drosophila melanogaster*, this phenomenon was dependent on the processing of the dsRNAs into fragments of ~21–25 nucleotides (MILESTONE 7). This observation was pivotal to the development, in 2001, of dsRNAs that could silence specific genes in mammalian cells.

Indeed, despite the efficiency of dsRNA-mediated gene silencing in insect cells, dsRNAs of 38–1,662 base pairs (bp) in mammalian cells did not seem to silence specific genes, perhaps because dsRNAs of >30 bp activate a cellular interferon response that triggers non-specific degradation of mRNA. In an attempt to circumvent this problem, Elbashir et al. worked downstream of the dsRNA-processing step by directly generating 21-bp small interfering RNA (siRNA) duplexes with symmetric, two-nucleotide 3′ overhangs. Transfecting siRNAs against a reporter gene into mouse, monkey and human cells repressed reporter gene expression by 2–25-fold, indicating that siRNAs function in mammalian cells. Substituting uridine with thymidine in the 3′ overhang, to protect the siRNA from nuclease degradation and to reduce the cost of siRNA synthesis, did not compromise knockdown (repression) efficiency or specificity. Furthermore, an siRNA concentration of just 1.5 nM, which was several orders of magnitude below the concentration of conventional antisense molecules required for gene silencing, could silence target genes. Together, these findings suggested that siRNAs were powerful tools for gene silencing in mammalian cells.

Comparing siRNAs with dsRNAs of 50 bp and 500 bp in gene targeting assays confirmed that the short length of siRNAs was key to gene-specific silencing in mammalian cells. Longer dsRNAs triggered the degradation of non-cognate reporter genes through the interferon response, rendering their effect on the target gene difficult to detect.

> “…this 2001 study uncovered the promise of siRNAs for the study of gene function and highlighted their potential utility for gene-specific therapies”

Credit: Lara Crow / Springer Nature Limited

The final confirmation that siRNAs could silence genes in mammalian cells came from the observation that, 40–45 hours after transfection, siRNA specifically reduced the endogenous expression of the gene encoding lamin A/C (by >90%) and of the genes encoding lamin B1 and nuclear mitotic apparatus protein (to low levels). Therefore, although the underlying mechanism was not yet clear, this 2001 study uncovered the promise of siRNAs for the study of gene function and highlighted their potential utility for gene-specific therapies.

The first proof-of-principle that siRNAs could be harnessed to treat disease came from a study published in 2003, showing that siRNAs could protect mice against fulminant hepatitis, which can result from excessive apoptosis in the liver. As hepatocytes express high levels of the FAS receptor, and are thus susceptible to FAS-mediated apoptosis, inhibiting *Fas* expression in hepatocytes was of interest for treating liver diseases.

Song et al. showed that tail vein injection of siRNA targeting *Fas* into mice could markedly reduce the expression of FAS at the gene and protein level compared with mice injected with *GFP* siRNA; these reductions were stable for 10 days after siRNA injection and were achieved at low doses. Importantly, whereas mice treated with *GFP* siRNA before or after injection with concanavalin A (to induce fulminant hepatitis) showed signs of liver damage, mice injected with *Fas* siRNA before or after concanavalin A treatment did not. Therefore, this study highlighted the potential of siRNAs in both preventing and treating liver injury.

The discovery that siRNAs could silence genes in mammalian cells, and indeed in whole mammalian organisms, revolutionized the study of gene function. Several advances in siRNA technology have been made in the years since their discovery, including tangible improvements in siRNA delivery (MILESTONES 11, 13), with the result that siRNAs are still widely used in the laboratory today.

Katharine H. Wrighton, Team Leader, Nature Reviews Cross-Journal Team

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MILESTONE 9

piRNAs — guardians of the germline

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By 2001, RNA interference — the sequence-specific inhibition of gene function by homologous double-stranded RNA (dsRNA) — had been observed in a wide range of eukaryotes. The phenomenon had been linked to transposon-repression and anti-viral defence, especially in plants, but the full spectrum and functional relevance of this mechanism in animals was unknown. Then, Gvozdev and colleagues demonstrated homology-dependent silencing of testis-specific Stellate genes mediated by small RNAs generated from both strands of the *Suppressor of Stellate* repeat locus in the *Drosophila melanogaster* male germline. Interestingly, relief of *Stellate* silencing also led to de-repression of retrotransposons and other genomic tandem repeats. This work marked the discovery of piRNAs, although it would take another five years until they gained this name.

In 2006, four studies used RNA sequencing to identify a class of 26–30-nucleotide-long RNAs that specifically associated with mammalian PIWI-clade Argonaute proteins in mouse, rat and human male germ cells — hence the name 'piRNAs', for PIWI-interacting RNAs. PIWI proteins had been genetically linked to germ cell and stem cell maintenance and to meiosis, although their biochemical function remained unknown.

At the time, the related AGO-clade subfamily of Argonaute proteins had been shown to act in RNA interference and microRNA-mediated gene regulation using 21–22-nucleotide RNAs as targeting guides. However, piRNAs seemed distinct. For example, there was little evidence for overlapping complementary RNAs or potential fold-back structures, suggesting that piRNAs might not be derived from dsRNA precursors. Zamore and colleagues then provided evidence that Dicer endonuclease activity — which is essential for microRNA and short interfering RNA biogenesis — was dispensable for piRNA generation in *D. melanogaster*. This finding led to the realization that piRNAs represented a novel class of Dicer-independent small silencing RNAs.

Yet, the mechanism governing piRNA biogenesis remained elusive until 2007, when two groups independently described an intricate piRNA amplification loop, the so-called 'piRNA ping-pong cycle'. Sequencing of small RNAs associated with all three *D. melanogaster* PIWI-clade proteins — Piwi, Aubergine (Aub) and Argonaute 3 (Ago3) — revealed that each protein binds to specific piRNA populations: Piwi-bound and Aub-bound piRNAs were mainly antisense to transposon sequences and harboured a strong preference for having a 5′ terminal uridine. Ago3-associated piRNAs, on the other hand, were biased for transposon sense strands and had a preference for an adenine at nucleotide 10, with no preference for uridine at the 5′ end. Most strikingly, the 5′ ends of Ago3-bound piRNAs were typically offset by precisely ten nucleotides from the 5′ ends of complementary Aub-bound piRNAs. This suggested a model in which an antisense piRNA, complexed with Aub, would recognize and cleave a sense transposon transcript. The cleaved product would then be processed into an Ago3-bound sense piRNA, which could seek out target transcripts. Ago3-directed cleavage triggers generation of the original antisense piRNA, capable of both silencing the target element and further amplifying the response. The majority of the initial antisense precursor RNAs were derived from discrete genomic loci, so-called 'piRNA clusters', which are comprised mainly of defective transposon sequences in the fly.

> "This finding led to the realization that piRNAs represented a novel class of Dicer-independent small silencing RNAs"

Together, these studies established the piRNA pathway as a transposon surveillance mechanism. Although a plethora of later studies provided additional exciting insights into the piRNA pathway and its function as a safeguard of genome integrity and fertility, many questions regarding the precise molecular mechanisms of piRNA generation and their diverse silencing functions are still unanswered and the subject remains an active area of research.

Anke Sparmann, Senior Editor,
Nature Structural & Molecular Biology

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MILESTONE 10

pSUPER start to large-scale RNA interference screening

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In 2002, Reuven Agami, Thijn Brummelkamp and René Bernards published two papers (in *Science* and in *Cancer Cell*) describing different versions of a plasmid-based expression system capable of driving continuous synthesis of a small interfering RNA (siRNA) in mammalian cells. These vector systems — pSUPER (suppression of endogenous RNA) and the retroviral version, pRETRO-SUPER — enabled targeted knockdown of gene expression with an efficiency matching that of synthetic siRNAs, yet without the loss of inhibition caused by prompt clearance of such siRNAs from mammalian cells, a disadvantage that had curtailed the applications of synthetic siRNAs.

Use of the pSUPER vectors achieved ~90% inhibition of the expression of the target gene and, in the case of pRETRO-SUPER, which integrates into the host genome, also enabled stable expression of the siRNA. These advances enabled the transfected cells to be cultured and the downstream effects of gene knockdown to be studied in detail.

Importantly, Brummelkamp et al. showed that RNA interference (RNAi) was highly sequence-specific: use of pRETRO-SUPER to deliver a *KRAS*-targeted siRNA inhibited the expression of mutant (oncogenic) *KRAS* mRNA without reducing the expression of the native mRNA. This specificity is essential for studying cancer, in which oncogenic and non-oncogenic alleles of the same gene (which might differ from each other by only a single nucleotide) are often co-expressed.

The pSUPER plasmid includes the promoter of H1 RNA polymerase III, placed upstream of a 19-nucleotide sequence derived from any gene of interest. This sequence is separated from its reverse complement 19-nucleotide sequence by a 9-nucleotide spacer, and is followed by a transcription termination sequence. Consequently, the RNA transcripts generated by pSUPER and pRETRO-SUPER self-fold into a stem–loop structure resembling a hairpin, and were therefore called short hairpin RNAs (shRNAs).

In their first 2002 paper, Brummelkamp et al. concluded that it should be possible to generate large collections of pSUPER shRNA vectors to carry out high-throughput genetic screens for loss-of-function phenotypes. Indeed, only two years later, the Bernards group demonstrated the utility of this system for large-scale screening. Berns et al. constructed a set of retroviral vectors encoding 23,742 distinct shRNAs, which targeted 7,914 human genes (that is, three different shRNAs targeting each gene) implicated in the promotion or suppression of cancer. The researchers then used this RNAi library to screen human cells and identified one known and five previously unknown components of the p53 tumour suppressor pathway, which induces cell cycle arrest, cell senescence or apoptosis. This study was the first to use vector-based shRNA libraries for large-scale functional genetic screening in human cells.

> “These vector systems… enabled targeted knockdown of gene expression with an efficiency matching that of synthetic siRNAs, yet without the loss of inhibition caused by prompt clearance of such siRNAs”

Other scientists were quick to realize the significance of this work. Agami, Brummelkamp and Bernards received thousands of requests for reagents from fellow researchers, and the use of siRNA-encoding (viral) vectors has since become a major method of gene suppression in mammalian cells.

Caroline Barranco, Senior Editor, Nature Reviews Cross-Journal Team

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MILESTONE 11

Targeted siRNA delivery in vivo

Advances in the characterisation of selective gene silencing through small interfering RNAs (siRNAs) increased the interest in using this approach as a therapeutic tool. The first proof-of-principle demonstration of the therapeutic potential of siRNAs was performed by Judy Lieberman's group in 2003 (MILESTONE 8). To silence gene expression in a mouse model, the group administered siRNA by hydrodynamic tail-vein injection, which has two major challenges for potential clinical application. First, it involves the exchange of a large proportion of the blood volume. Second, the method worked for liver cells but it was unclear if it could also be used to target other cells, for example, immune cells.

Both challenges were addressed by Lieberman and colleagues in a subsequent study published in 2005. To target specific cells in the body, the authors used an antibody fragment (F105) that recognised HIV-1 envelope protein, which is expressed on the surface of cells that are infected with HIV. They fused F105 with protamine, generating F105–P molecules. The fusion to protamine was necessary for binding siRNAs, because the antibody fragment itself does not bind them. F105–P had previously been shown to transport DNA into cells.

The researchers first confirmed that F105–P enabled the uptake of siRNA, and the selective and dose-dependent reduction of target mRNA levels only in cells expressing HIV-1 envelope. Then, they tested how F105–P works in primary T cells, which are challenging to transfect with conventional methods. F105–P loaded with siRNAs targeting the HIV-1 capsid gene *gag* not only decreased HIV replication in HIV-infected T cells, but also reduced the release of viral particles into the cell culture medium. This demonstrated that the approach enables the targeting of selected cell types based on cell surface proteins to silence or reduce mRNA expression.

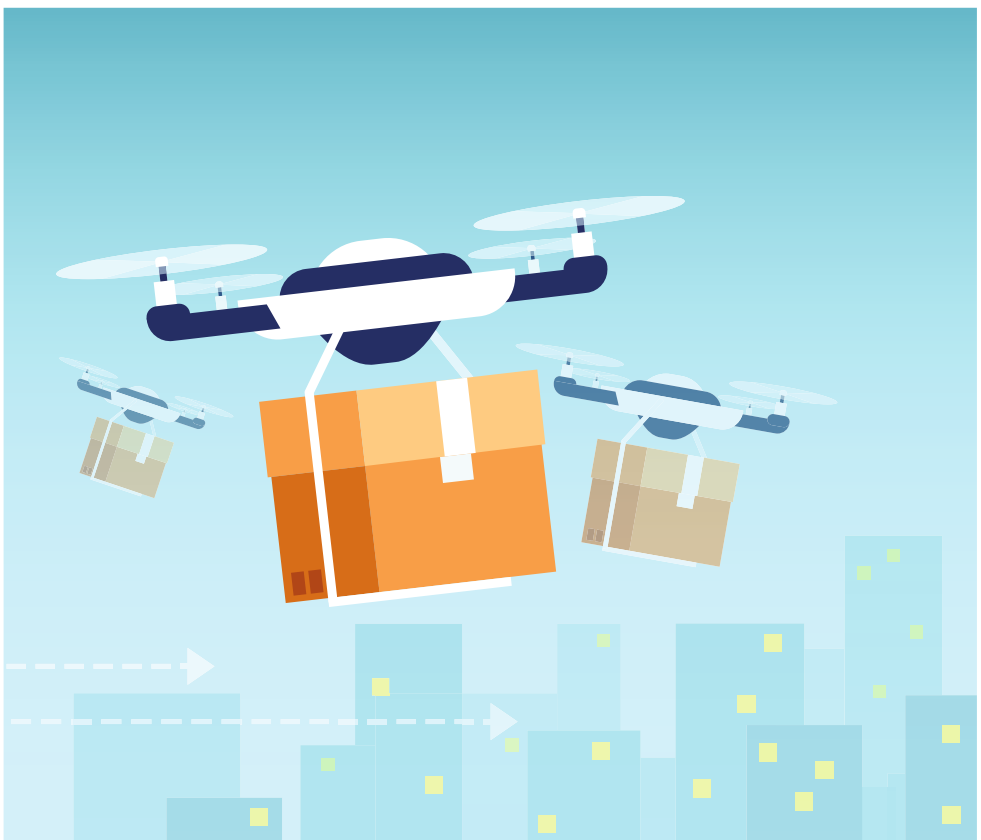
> “…targeted siRNA uptake by selected cells was an important step towards developing siRNAs for clinical applications…”

The next step was to show that F105–P-mediated gene silencing also works in vivo after systemic application of F105–P. For this, tumour cells expressing an HIV envelope protein were implanted into mice. Only these cells, but not surrounding cells, took up siRNA when F105–P was injected into the tumour cells or administered through intravenous injection. Furthermore, injection of F105–P molecules binding a mixture of siRNAs against tumour-growth-related genes reduced tumour growth, thereby demonstrating effective in vivo gene silencing by F105–P-mediated siRNA delivery.

The researchers also demonstrated the broader applicability of their approach by using an antibody against the receptor ERBB2 to silence Ku70 expression in *ERBB2*-expressing cells.

The major achievement of this work was the proof-of-principle demonstration of targeted siRNA delivery in vivo. A similar approach, published a year later, used RNA-based aptamers instead of antibodies, which are easier to produce and less immunogenic. Both methods laid the foundation for targeted siRNA delivery in vivo. Although some important issues, like the pharmacokinetics and a detailed safety and toxicity assessment, remained to be investigated in more detail, targeted siRNA uptake by selected cells was an important step towards developing siRNAs for clinical applications while minimising adverse side effects.

Christian Schnell,
Associate Editor, *Nature Communications*



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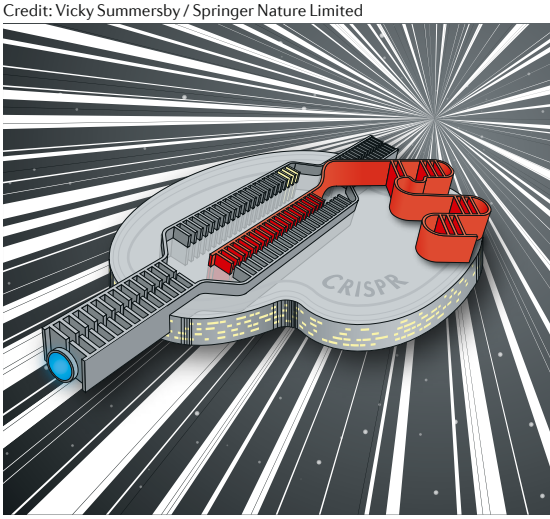
MILESTONE 12

Gene editing by CRISPR–Cas

Credit: Vicky Summersby / Springer Nature Limited



One of the most revolutionary developments in biology has its origins in the RNA-based defence system of bacteria, which encodes clustered regularly interspaced short palindromic repeats (CRISPR) along with CRISPR-associated (Cas) proteins. CRISPR–Cas has been adapted to function as a programmable genome-engineering tool that has enabled easy targeting and manipulation of precise genomic sequences in bacteria, plants, fungi and mammals, including humans.

Prior work with programmable genome-engineering tools focused on the use of various nucleases for gene targeting. Though precise, the reliance on protein-based recognition of a target DNA sequence meant that each new target required redesigning the tool, which is often a laborious task. CRISPR–Cas has the advantage of being precise and highly adaptable. This precision is derived from base-pairing between the complementary CRISPR–Cas guiding RNA and target DNA, which creates a straightforward and easy-to-adapt targeting system.

Foundational work was performed in 2012 by groups studying the function of CRISPR and the associated protein Cas9 in bacteria. It was known that these CRISPR–Cas systems function as a bacterial immune system against viruses. Jinek et al., and complementary work from Gasiunas et al., demonstrated that two small RNAs — the CRISPR RNA (crRNA) in a complex with a *trans*-activating CRISPR RNA (tracrRNA) — function together in targeting the nuclease Cas9 to specific DNA sequences, where it can generate a double-stranded DNA break (DSB). Jinek et al. had the insight that the two RNAs could be joined together to form a single guide RNA (sgRNA). The groups speculated that RNA could also be used to guide Cas9 to specific genomic sequences and thus enable gene editing and genome engineering.

The concept was demonstrated in a set of papers published in early 2013, when Jinek et al., Mali et al. and Cong et al. adapted the bacterial CRISPR–Cas system to function in mouse and human cells. Using sgRNAs and optimising the system for expression in a mammalian context, they demonstrated that Cas9 could be guided to specific target sequences in the larger and more complex mammalian genomes where, as in bacteria, it cleaved the DNA.

Mammalian cells attempt to rapidly repair these DSBs and this process can be co-opted for tailored genome engineering. The non-homologous end-joining DSB repair pathway attempts to stitch the DNA ends back together, often resulting in the introduction of deleterious mutations in genes. Furthermore, if a

MILESTONE 13

Chemical optimization improves oligonucleotide delivery

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Forty years after their initial discovery, oligonucleotide therapeutics had begun to show potential to reach clinical maturity. By 2014, several drugs had been approved for disease treatment, and many others were undergoing clinical trials at various stages. However, being able to deliver effective doses of oligonucleotides to a specific set of diseased cells or organs was still challenging.

Small interfering RNAs (siRNAs) had been successfully used to inhibit the expression of disease-causing genes, and the implementation of antibody-mediated and lipid-nanoparticle-mediated delivery had significantly improved their efficiency. However, these treatments often required high doses and repeated intravenous injections to be therapeutically effective, which limited their clinical applicability in cases where intravenous drug administration was not feasible. Therefore, researchers worked to develop chemically modified oligonucleotides to enable efficient delivery to target cells by subcutaneous administration.

How such chemical optimisation could improve siRNA delivery was demonstrated in 2014 by Manoharan and colleagues, who covalently conjugated siRNA to *N*-acetylgalactosamine (GalNAc), a ligand of the asialoglycoprotein receptor (ASGPR). This receptor is expressed on the surface of liver cells and is responsible for the uptake of circulating glycoproteins with exposed GalNAc glycans. This design enabled targeted siRNA–GalNAc delivery to the liver owing to the high-affinity binding between receptor and ligand. The first tests were performed in cultured mouse liver cells and showed that receptor binding-affinity correlated with siRNA uptake efficiency. Encouraged by these results, the researchers tested the ability of siRNA–GalNAc conjugates to silence gene expression in vivo. They observed a robust and durable silencing of the targeted gene, transthyretin, in the liver of mice after a single or multiple low-volume subcutaneous administrations. The extent of silencing was higher following subcutaneous administration compared with intravenous administration.

In a study published a year later, the same research group optimised the design of the conjugates to improve therapeutic effectiveness. The researchers tested various attachment sites for the GalNAc ligand on the RNA molecule, and evaluated silencing activity both in vitro and in vivo. They found that placing three monovalent GalNAc units in close proximity to each other

> …CRISPR–Cas has opened up new avenues in our understanding of how cells repair DNA damage, in our ability to engineer cells and in the possibility of developing new, RNA-dependent therapies for previously intractable genetic diseases

DNA template is available the cell will attempt to copy genetic information from it during repair, thereby inserting new genetic information into the genome, as demonstrated by Mali et al. and Cong et al.

From its origins as a bacterial immune system, CRISPR–Cas has been developed into an all-purpose tool for tailored engineering of genomes in a range of species. In the span of less than a decade, CRISPR–Cas has opened up new avenues in our understanding of how cells repair DNA damage, in our ability to engineer cells and in the possibility of developing new, RNA-dependent therapies for previously intractable genetic diseases.

Ross Cloney,
Senior Editor, *Nature Communications*

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> This seminal work established siRNA–GalNAc as a promising therapeutic delivery approach to treat diseases involving liver-expressed genes

on the RNA sense strand resulted in a higher-affinity binding to ASGPR on liver cells, and a more robust silencing in vivo.

This seminal work established siRNA–GalNAc as a promising therapeutic delivery approach to treat diseases involving liver-expressed genes. Despite halting of the development of the first siRNA–GalNAc-based drug (Revusiran) during clinical trials in 2016, the impressive silencing efficiency, good safety profile and encouraging results from more recent clinical trials of drugs for acute hepatic porphyria (Givosiran) and cardiovascular disease with elevated LDL cholesterol (Inclisiran), established GalNAc conjugation as a promising solution for therapeutic siRNA delivery to the liver.

Alfredo Sansone,
Senior Editor, *Nature Communications*

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MILESTONE 14

An antisense oligonucleotide splicing modulator to treat spinal muscular atrophy

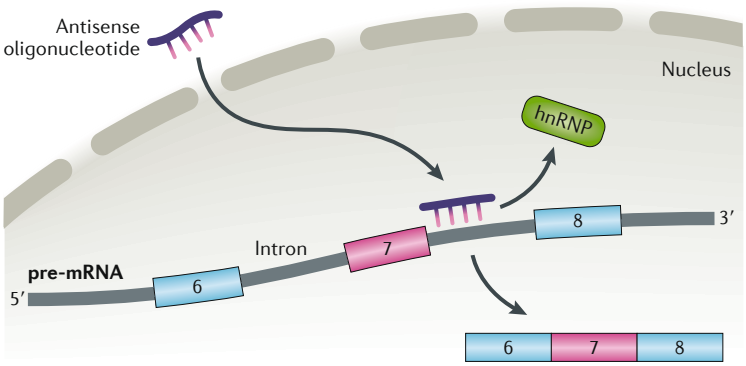

Figure adapted with permission from Rigo, F. et al. *J. Cell Biol.* **199**, 21–25 (2012)

On 23 December 2016, the United States Food and Drug Administration (FDA) approved the antisense oligonucleotide (ASO) drug nusinersen (Spinraza) to treat spinal muscular atrophy (SMA), a fatal genetic disease that can affect children and adults. The approval was the culmination of a successful collaboration between researchers in academia and industry, with support and assistance from patient advocacy groups and regulatory agencies.

SMA is a devastating neuromuscular disease that affects 1 in 10,000 people and is caused by mutations in the gene survival of motor neuron 1 (*SMN1*). Without functional SMN protein, the motor neurons in the spinal cord and brain stem degenerate, resulting in muscle weakness and atrophy. Of the infants born with SMA, 60% show symptoms before six months of age, with median life expectancy of less than two years. A paralog of *SMN1* in the human genome, *SMN2*, encodes an identical SMN protein. However, its pre-mRNA undergoes aberrant splicing, with 90% of mature *SMN2* transcripts lacking exon 7 and producing a truncated, unstable polypeptide. Some individuals with SMA carry multiple copies of *SMN2* and can thus produce higher levels of full-length SMN protein, which reduces the severity and delays the onset of the disease.

The molecular basis of *SMN2* exon 7 skipping was elucidated by several groups, including those of Ravendra Singh at University of Massachusetts Medical School and Adrian Krainer at the Cold Spring Harbor Laboratory, in the late 1990s to early 2000s. *SMN2* contains a synonymous C-to-T substitution in exon 7 that weakens the binding of splicing activators, thereby reducing the efficiency of the 3′ splice site. In 2003, Cartegni and Krainer engineered bifunctional ASOs that operate as synthetic splicing activators: a peptide mimicking a splicing activator was covalently linked to an ASO that hybridized to exon 7. This chimeric effector was able to promote exon 7 inclusion in cell extracts. Those findings prompted C. Frank Bennett, from Isis (later Ionis) Pharmaceuticals, to contact Krainer and initiate a collaboration, as recounted by Rigo et al. in 2012.

Over the next years, the strategy to control exon 7 inclusion was optimized for use in cells and

animal models. ASOs targeting a site near the 5′ splice site in *SMN2* intron 7 could efficiently promote exon 7 inclusion without the need of an appended peptide moiety. They acted by preventing binding of the splicing repressors HNRNPA1 and HNRNPA2. In addition, chemical modifications in the backbone (phosphorothioate) and nucleotides (2′-*O*-methoxyethyl, or 2′-MOE) of the ASOs improved their pharmacological properties.

With promising results in pre-clinical studies, the ASO nusinersen entered clinical trial phase I and II studies in 2011 and 2013–2014, respectively. A multi-centre, randomized, double-blind phase III study took place in 2014–2016 and included 121 infants up to seven months of age who had been diagnosed with SMA before they were six months old (infantile onset). Participants received the drug injected intrathecally (that is, through a lumbar puncture for delivery into the cerebrospinal fluid, to reach targets in the central nervous system). A control group was treated with a mock procedure (skin prick). An interim analysis conducted with 82 patients showed that 40% of those treated with nusinersen showed improvements in motor function milestones, such as head control, sitting, rolling, crawling, standing and walking, compared to none in the control group. These results led to early termination of the trial in August 2016, so that infants in the control group could start receiving the drug. Beneficial effects observed in another trial, with children aged 2–12 with later-onset SMA, also prompted its early termination in November 2016.

> “…a successful collaboration between researchers in academia and industry, with support and assistance from patient advocacy groups and regulatory agencies”

The FDA approved nusinersen only three months after the new drug application (NDA) was filed by Biogen. This occurred with fast-track designation and priority review, and without an advisory committee, as outside expertise was not deemed necessary given the lack of controversial issues, as noted in the agency's summary report. Nusinersen was approved by the European Medicines Agency in May 2017, and it is currently available for treating SMA in more than 40 countries.

Inês Chen, Chief Editor,
Nature Structural & Molecular Biology

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MILESTONE 15

A new dawn for RNAi drugs

The 10th of August, 2018 marked a new era for the field of RNA therapeutics, with the first approval of an RNA interference (RNAi)-based drug by the United States Food and Drug Administration. The drug — patisiran (Onpattro) — is approved for the treatment of polyneuropathy in people with hereditary transthyretin-mediated amyloidosis (hATTR). This rare and devastating neurodegenerative disease is caused by deposition of amyloid fibrils formed by misfolded transthyretin protein. A double-stranded small interfering RNA composed of two modified 21-mer oligonucleotides and encapsulated in a lipid nanoparticle formulated for hepatocyte uptake, patisiran silences transthyretin mRNAs in the liver to reduce serum levels of the protein. The approval of patisiran brings new hope to patients with hATTR who previously had no effective treatment options.

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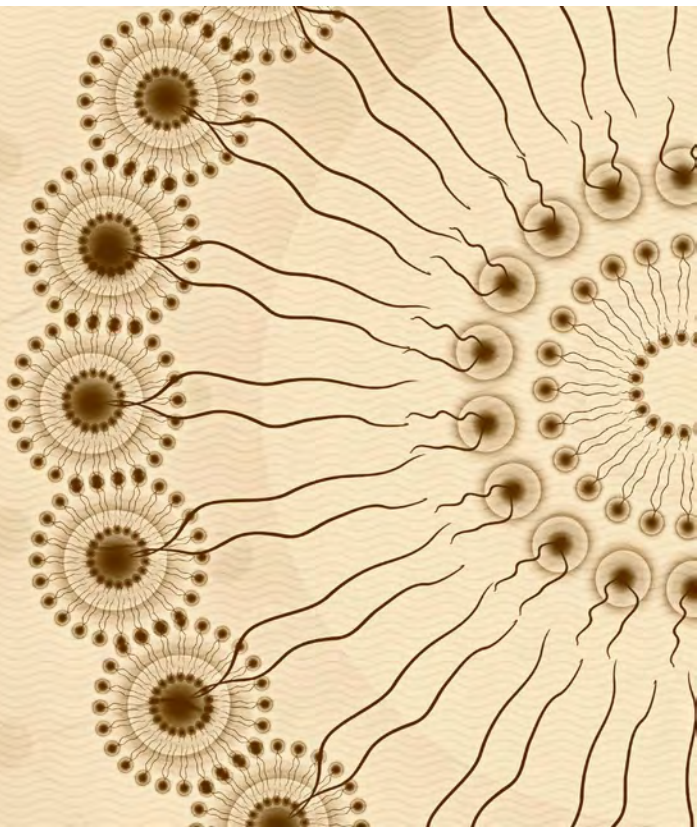

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