lutionary advantage" as delivery vehicles for small RNAs, he adds.

By exploiting the natural diversity of viruses, researchers have developed a suite of viral vectors that can be used to deliver therapeutic sequences into a wide variety of cell types. Viral vectors have been around for some time and scientists are currently using them to deliver short hairpin RNA (shRNA) that can be processed by cells to yield siRNAs and silence genes.

Viral vectors from the DNA-based adenovirus and adeno-associated virus (AAV) have been used by researchers and companies, such as ArmaGen Technologies in Santa Monica, California, for shRNA delivery. AAV vectors have, however, found more widespread use in RNAi applications as these viruses can transduce both dividing and non-dividing cell types and result in stable, site-specific integration.

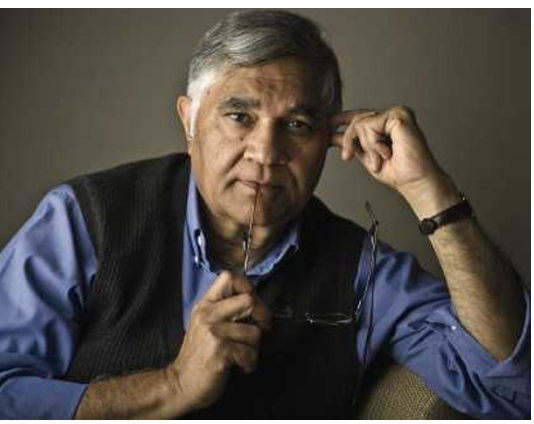
Lentivirus-based vectors are another option. "The utility of lentiviral vectors for RNAi research is their ability to transduce non-dividing cells and to efficiently transduce difficult-to-transfect cells," says Boro Dropulic, founder and chief executive of Lentigen in Baltimore, Maryland.

But any viral vector will still have to overcome the issue that plagues synthetic RNAs: targeting. "Targeting is a big problem. There are several groups working on that issue now but the jury is still out," says Dropulic. What makes targeting viral vectors such a challenge is the difficulty in altering the viral structure with targeting elements while maintaining the proper function of viral particles.

For adenovirus and AAV vectors, for example, the viruses require a specific number of proteins to make their viral shell. Adding more proteins, such as an antibody fragment for targeting purposes, could be problematic. "Imagine the viral shell is like the dome of a sports centre," says Verma. "You can only put so much information in there, after which the dome will break." To overcome this problem, Verma thinks that researchers will need to find a way to modify the entire shell of the virus.

Lentiviral vectors are less problematic — the virus particles are surrounded by a lipid envelope in which proteins can be inserted without disrupting the viral structure. Even so, efficient and specific targeting remains an issue.

I. VERMA



Inder Verma believes viruses have an evolutionary advantage for RNAi delivery.

MERCK

Sirna Therapeutics is developing stable siRNA compounds for silencing disease-related genes.

"I think that it will come down to two things: a more extensive learning of the modification of the viral structure proteins, and identification of specific target receptors so that you can have the viruses interact with cells in a very specific manner," says Verma. He points to recent work from David Baltimore's lab at the California Institute of Technology in Pasadena, in which a small monoclonal antibody was linked to a lentiviral envelope protein for targeting to specific cell types[4], and to other groups trying to use small ligands and signature sequences from cell-surface receptors as promising developments for the targeting of viral vectors.

As researchers wrestle with the targeting issue, some companies have already begun to use lentiviral vectors to deliver therapeutic RNAi. Dropulic sees the *ex vivo* use of lentiviral transduction systems as being a critical first step. "I think using the transduced cells as the vehicles of widespread dissemination rather than thinking about direct injection of lentiviral vectors will be the way to go," he says. Lentigen is using this approach on T cells and stem cells for cancer therapies as well as for treatment of infectious diseases.

The silent future

Most experts agree that therapeutic RNAi will probably not rely on a single delivery vehicle or administration approach for all diseases. "Some diseases might require lower doses than others or could be semi-local in nature," says Robert Langer, a bioengineer at MIT, "so I think a lot may depend on the disease and treatment modality you are thinking about."

One likely beneficiary of the work on delivery mechanisms are siRNA's recently uncovered siblings: microRNAs (miRNAs), which are naturally occurring siRNAs found in plants and animals. "With miRNA therapeutics, you are generally focusing on a new class of non-coding RNAs where the biology is still being discovered," says Maraganore. It is early days for this technology, but companies are now being established to work exclusively on miRNA-based therapies (see 'Thinking small').

When it comes to siRNA, Maraganore is encouraged by the early approaches to delivery being used in clinical studies. He expects the development of new delivery vehicles to continue for sometime to come. "I suspect that in 35 years scientists will continue to work on optimizing delivery approaches," he says.

And Verma thinks that the discovery of RNAi has even breathed fresh life into the still struggling field of gene therapy. "I think having RNAi technology available now gives a new impetus because it is such an effective technology — that is, if only we could deliver it." ■

Nathan Blow is technology editor for *Nature* and *Nature Methods*.

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Correction

In the Technology Feature 'The personal side of genomics' (*Nature* **449,** 627–630; 2007) we said that Illumina uses emulsion PCR in its next-generation sequencing systems. In fact, the company's genome analyser uses solid-phase amplification on a planar, optically transparent surface.