

of nanometres^{8,9}. With metastable atoms it may be possible to create more complex patterns using atom holography¹⁰. The efficiency of these processes, although currently low, could be enhanced significantly with an efficient 'atom laser' constructed from a metastable BEC.

So although Bose–Einstein condensates of He* and liquid helium are composed of the same atomic element, they have very different characteristics. The unique properties of a BEC of metastable helium gas will give researchers a powerful tool with which to learn more about this fascinating state of matter.

Randall G. Hulet is in the Department of Physics

and Astronomy and Rice Quantum Institute, Rice University, Houston, Texas 77251, USA. e-mail: randy@atomcool.rice.edu

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RNA interference

The short answer

Brenda L. Bass

One way of seeing what a gene does is to block its messenger RNA and note the effects. New work should make the approach more broadly applicable.

RNA interference (RNAi) was discovered only a few years ago¹, but many scientists find it hard to imagine life without it. Once the sequence of a gene is known, RNAi offers a quick and easy way to determine its function, and the technique is accessible to a scientist in a small lab, as well as to a consortium attempting to assign function to the genes of an entire chromosome^{2,3}. But although RNAi is now routine in laboratories studying a wide range of organisms, its use in mammalian cells has been problematic. On page 494 of this issue⁴ Tuschl and colleagues describe research that paves the way for successful RNAi in mammalian cells.

The basic idea behind RNAi is shown in the right-hand part of Fig. 1 — this is the sequence-specific pathway indicated by blue arrows. A double-stranded RNA (dsRNA) matching a gene sequence is synthesized *in vitro* and introduced into a cell. The dsRNA feeds into a natural, but poorly understood, biological pathway, and is broken into short pieces called short interfering (si) RNAs⁵. With the help of cellular enzymes that have not yet been well characterized⁶, the siRNA triggers the degradation of the messenger RNA that matches its sequence. This often leads to adverse consequences for the organism, evident in an aberrant phenotype, that allow the gene's function to be identified.

RNAi was first discovered in the nematode worm *Caenorhabditis elegans*¹, but is present in many other organisms (the fruit fly *Drosophila*, certain parasitic protozoa, and plants, for instance), and so seems to represent an ancient pathway⁷. Nonetheless, researchers have always been pessimistic

about applying RNAi to mammalian cells, because exposing such cells to dsRNA, of any sequence, triggers a global shut-down of protein synthesis⁸. This nonspecific pathway is indicated on the left of Fig. 1 by a red arrow. The lore has been that this pathway would mask any sequence-specific effects that might occur from the RNAi pathway.

But it almost always pays to consider how one's own research fits in with previous observations. Tuschl and colleagues were, it seems, being especially diligent in this respect. Their earlier work showed that the

siRNA intermediates themselves could initiate RNAi, at least in non-mammalian cells⁵. However, the nonspecific pathway requires longer dsRNA, and will not occur with dsRNAs shorter than around 30 base pairs^{8–10}. They don't say as much in the paper, but one presumes that Tuschl's group began with the idea that, because of this size discrimination, siRNAs might be able to bypass the more global, nonspecific response. They turned out to be right.

First, Tuschl and colleagues⁴ tested whether siRNAs could trigger RNAi in mammalian cells, as had been observed in non-mammalian cells. They assayed the ability of siRNA to target various luciferase transgenes, for which gene expression is easily quantified by measuring luminescence. siRNAs were transfected with cationic liposomes into various mammalian tissue culture cells (NIH/3T3, COS-7, HeLa and 293 cells), as well as into a *Drosophila* cell-culture line for comparison. Indeed, the authors observed reproducible, sequence-specific siRNA inhibition in the mammalian cells, with no sign of the nonspecific effects. In contrast, with longer RNAs, luciferase expression was reduced with every dsRNA tested, no matter what its sequence. Superimposed on the nonspecific inhibition was a sequence-specific inhibition, suggesting that both pathways can operate simultaneously. (As shown in Fig. 1, the two pathways probably compete for the long dsRNA.) Importantly, Tuschl and co-workers went on to show that siRNAs are not only effective at targeting the transgene luciferase, but also at targeting naturally occurring, endogenous genes.

Of course, the story is not as neat and tidy as I have described it here. As the authors are

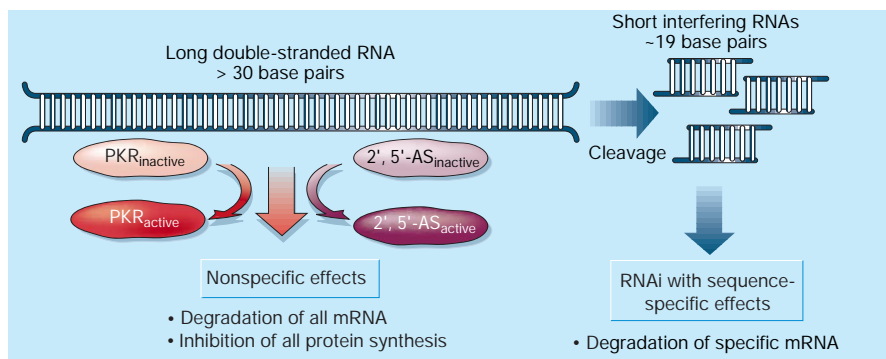


Figure 1 Mammalian cells have at least two pathways that compete for double-stranded RNA (dsRNA). In the RNAi, or sequence-specific, pathway (blue arrows), the initiating dsRNA is first broken into short interfering (si) RNAs. siRNAs have sense and antisense strands of about 21 nucleotides that form 19 base pairs to leave overhangs of two nucleotides at each 3' end. siRNAs are thought to provide the sequence information that allows a specific messenger RNA to be targeted for degradation. The nonspecific pathway (red arrow) is triggered by dsRNA of any sequence, as long as it is at least 30 base pairs long. The nonspecific effects occur because dsRNA activates two enzymes: PKR, which in its active form phosphorylates the translation initiation factor eIF2a to shut down all protein synthesis, and 2', 5' oligoadenylate synthetase (2', 5'-AS), which synthesizes a molecule that activates RNase L, a nonspecific enzyme that targets all mRNAs. The nonspecific pathway represents a host response to stress or viral infection; in the second case, the activating dsRNA is thought to derive from viral replication.

careful to point out, inhibition by siRNAs is effective in mammalian cells, but gene expression is not eliminated completely as it is in *Drosophila* cells. Further, siRNA techniques in mammalian cells have some of the same drawbacks associated with antisense RNA, another technique used to prevent expression of particular genes. In both cases, success depends on the cell type, as well as on the level of expression of the gene to be targeted. That apart, however, RNAi has repeatedly proven itself to be more robust than antisense techniques: it works more often, and typically decreases expression of a gene to lower levels, or eliminates it entirely. And, as Tuschl and colleagues show, even in mammalian cells, siRNAs are effective at concentrations that are several orders of magnitude below the concentrations typically used in antisense experiments.

One of the most important aspects of the new work is the further research it will inspire. Although RNAi works in mouse eggs and embryos^{11,12}, scientists have been reluctant to invest time in applying it to other mammalian cells because of reported problems^{13,14}. Now we will see studies aimed at optimizing the use of siRNAs, as well as at understanding why conventional RNAi, with longer dsRNA, works in eggs and embryos. Might these cells lack the non-specific pathway?

The RNAi technique has had a huge impact in studies of non-mammalian systems. Use of siRNA in mammalian cells could be just as far-reaching, with the applications extending to functional genomics and therapeutics. But various technical issues must be addressed, especially for large-scale applications. For instance, dsRNA can be delivered to *C. elegans* by feeding or soaking, but effective delivery of siRNAs to mammalian cells will not be so simple. The analysis of *C. elegans* phenotypes is aided by short generation times and a wealth of information about the worm's morphology and behaviour; developing rapid ways to screen mammalian cells, or whole organisms, will take some time and thought.

So far I have discussed RNAi as a technique. But of course the pathway does not exist in cells solely to make life easier for scientists. RNAi is a natural biological pathway, albeit one we don't quite understand yet. Especially for those with a long-standing interest in the roles of dsRNA, Tuschl and colleagues' paper is interwoven with information about how RNAi coexists with previously characterized dsRNA pathways. This is especially interesting because dsRNA-binding proteins are usually not sequence specific and will bind any dsRNA. A single dsRNA can interact with proteins of different pathways so that the pathways compete. The different ratios of specific to nonspecific inhibition observed by the authors are probably telling us something about the particu-

lar constellation of dsRNA-binding proteins in the different cell types and how they compete with RNAi. Regardless of that, the new study shows that one way dsRNA pathways can coexist is to require different lengths of dsRNA. This is good news for cells — and for researchers. ■

Brenda L. Bass is in the Department of Biochemistry and Howard Hughes Medical Institute, University of Utah, 50 North Medical Drive, Room 211, Salt Lake City, Utah 84132, USA. e-mail: bbass@howard.genetics.utah.edu

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Chemistry

Synthetic lessons from quinine

Steven M. Weinreb

As the oldest, naturally occurring, treatment for malaria, quinine has been a target for synthetic chemistry for 150 years. At last, modern techniques provide full control over the synthetic molecule.

Nowadays the isolation and structure determination of a new biologically significant, naturally occurring molecule stirs the interest of synthetic chemists as well as biologists. In many cases, such a discovery is quickly followed by a laboratory synthesis. Organic chemists now have the ability and tools to synthesize virtually any large and complex natural product, but there are a few 'classical' compounds that have been revisited by the synthetic community time and time again. One such compound is the alkaloid quinine, chiefly used to treat malaria. In the *Journal of the American Chemical Society*, Gilbert Stork and co-workers¹ show that chemical 'firsts' are still possible — even when dealing with a natural product that has been used in medicine for over 300 years, and which has been a prime target for synthetic organic chemists for 150 years.

The Countess of Chinchon, the consort of the Spanish Viceroy of Peru, first popularized quinine as a treatment for malaria in the early seventeenth century. Natural quinine and related alkaloids are extracted from the bark of *Cinchona* trees. These alkaloids were first isolated in pure form in the early nineteenth century, but the structures of the major compounds were not worked out for another 100 years. Like many drugs, quinine (C₂₀H₂₄N₂O₂) can exist in several structural forms. It contains 'asymmetric' carbon atoms, which are connected to four different chemical groups and so allow the overall structure to be arranged in different ways. For quinine, 16 such stereoisomers are possible, but only one corresponds to the active form of the drug (Fig. 1, overleaf).

Because the supply of quinine depended

on the political vagaries of the producing countries — especially during wartime — organic chemists soon became interested in synthesis as a way to ensure a constant supply of the drug. Early attempts to synthesize quinine are legendary. In 1856, even before the structure of quinine was elucidated, William Henry Perkin² naively attempted to prepare the alkaloid by oxidizing allyltoluidine (C₁₀H₁₃N). In the course of this work he inadvertently produced the first synthetic dye, which was the main event responsible for the founding of the organic chemical industry.

When detailed structural information on the *Cinchona* alkaloids became available in the twentieth century, more rational approaches to quinine synthesis were devised. In 1944, two Harvard chemists, R. B. Woodward and William Doering, announced the first formal synthesis of quinine³, despite the limited synthetic and spectroscopic tools available at that time. Although they did not actually make quinine itself, they succeeded in synthesizing an intermediate compound known as homomeroquinene (Fig. 1). This compound is a degradation product of quinine, and had reportedly been converted back to the alkaloid by Paul Rabe in 1918 (although that account is now in dispute)^{1,4}. So Woodward and Doering had developed a synthetic route to quinine insofar as they assumed that Rabe's part of the sequence would work. But their synthesis of homomeroquinene was inefficient, largely because they could not control the spatial arrangement of atoms around the asymmetric carbons. This meant they actually generated several stereoisomers of homomeroquinene, which then