

## EDITORIAL



## Therapeutic RNA?

The genome of the majority of viruses of medical importance is comprised of ribonucleic acid (RNA), and this remarkable molecule is also a fundamental component of cells, in which it occurs in three major forms as ribosomal, transfer and messenger RNA, all of which function in the biosynthesis of proteins. Messenger RNA (mRNA) carries the coding information which is translated into protein, and many years ago it was shown that the complement of a mRNA, when hybridised with it to form a double strand, prevented translation of the mRNA into protein. Using *in vitro* cell-free translation systems, this technique, which became known as hybrid-arrested translation, was first employed in the assignment of protein coding functions to the individual segments of influenza virus genome RNA [1].

In the last three years, it has become apparent, initially through work on the nematode worm *Caenorhabditis elegans* and the fly *Drosophila melanogaster* that genes may be silenced *in vivo* by small interfering RNAs (siRNAs) which are complementary in sequence to mRNA [2,3]. The siRNAs are normally double-stranded RNA (dsRNA) molecules of <30 base-pairs(bp) which are generated from longer dsRNAs by a cytoplasmic ribonuclease enzyme known as Dicer. These siRNAs are then incorporated into a silencing complex called RISC (RNA-induced silencing complex) which identifies and silences complementary mRNAs.

However the direct introduction of dsRNAs >30 bp into mammalian cells in culture activates an interferon response with non-specific mRNA degradation and inhibition of protein synthesis which prevents their use in these cells. Fortunately, small ds RNAs of 20- to 23 bp do not cause an interferon response, so they can be used to cause sequence-specific silencing of gene expression [4,5]. Another method of generating siRNAs is by enzymatic engineering of DNA, for example a double-stranded complementary DNA (cDNA) library, to produce short (20 bp) fragments of hairpin RNA (shRNAs) which are cloned into a

retroviral expression vector to yield multiple siRNAs in cells over a long period from the integrated retroviral vector [6]. A number of different techniques may be used in mammalian cells [7,8], and other virus vectors including herpes simplex virus [9] and simian virus 40 pseudovirions [10] have been successfully employed as delivery systems. So what are the prospects for using siRNAs to control virus infections of medical importance?

Obvious first targets were viruses for which vaccines are not yet available, and it has now been shown that human immunodeficiency virus type 1 replication [11,12], hepatitis C virus expression [13], both acute and chronic infection with the prototype arenavirus, lymphocytic choriomeningitis virus [14], and the replication of human coronavirus SARS in rhesus macaques [15] are all susceptible to inhibition by sequence-specific siRNAs. The recent availability of a sound cell culture system for hepatitis C virus replication [16] will facilitate studies on siRNA as a potential therapeutic treatment for hepatitis C.

A number of obstacles will need to be overcome before siRNAs can be brought to clinical trial. For highly mutable viruses such as HIV, the high sequence specificity of RNA interference may result in rapid generation of resistant mutants, so that several viral targets, or essential host genes, may need to be inhibited if the siRNA treatment is to be effective [17]. There is also a need to work out effective delivery systems, so that siRNAs can reach their target cells, but the potential use of siRNAs in gene therapy of other diseases is accelerating progress in this field [18].

Finally, it now seems likely that RNA interference may prove an important therapeutic approach to pursue in the face of pandemic influenza. As we showed back in 1977 [1] it is quite easy to prevent the expression of influenza virus genes by RNA interference, and work in influenza virus-infected mice has shown that siRNAs can be used both to prevent and to treat influenza virus infection in this model system [19]. The siRNAs

can be given directly by intravenous injection, or intravenously or intranasally as DNA vectors from which siRNA precursors can be transcribed. The prospects for a new approach to the control of influenza in human populations appear ripe for exploration.

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