MicroRNAs

Small players in big diseases

Sheila Graham

Small molecules called miRNAs play a key role in development. miRNAs have been the focus of new research owing to the discovery that they play a role in cancer and other diseases. RNA biologist Sheila Graham explains what microRNAs are, what they do and how they might be used as therapies for disease

A dividing cancer cell

Key words
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Ribonucleic acid (RNA) is a nucleic acid present in all cells. It is similar in structure to DNA but while DNA occurs as a fairly rigid double helix, RNA is a flexible molecule that can form both simple and intricate shapes and structures.

Until recently, scientists thought there were just two main types of RNA in cells. First, RNA can be a structural molecule. It can act as a scaffold for building ribosomes that carry out protein synthesis in cells. Ribosomes are made up of ribosomal RNA — a very long nucleic acid — and a number of ribosomal proteins. The second and perhaps best understood role of RNA in the cell is as messenger RNA (mRNA). When genes are expressed, the genetic information encoded by DNA is copied into RNA in a process known as transcription. The mRNA is then translated or decoded to produce proteins — the building blocks of cells (see Figure 1 and pp. 2–5, this issue).

A new RNA

RNA molecules that are too small to have any structural significance and that do not act as mRNA have been discovered recently. These RNAs are called microRNAs (miRNAs). As the name suggests, miRNA molecules are very small. The average mRNA is 100 times the length of the average miRNA.

These ultra-short RNAs are found in plants and animals and in some viruses. They were first discovered in a nematode worm called *Caenorhabditis elegans* (*C. elegans*), which lives in the soil. The first miRNA to be discovered in *C. elegans* in 1993 was called *lin-4*. This molecule prevents the synthesis of a protein called LIN-14, which is responsible for the development of the worm larvae. However, at the time of discovery the researchers did not understand the far-reaching consequences of finding *lin-4*, for example that *lin-4* might have roles other than controlling development and may have roles in animals other than nematodes.

Light micrograph of *Caenorhabditis elegans* (× 120)

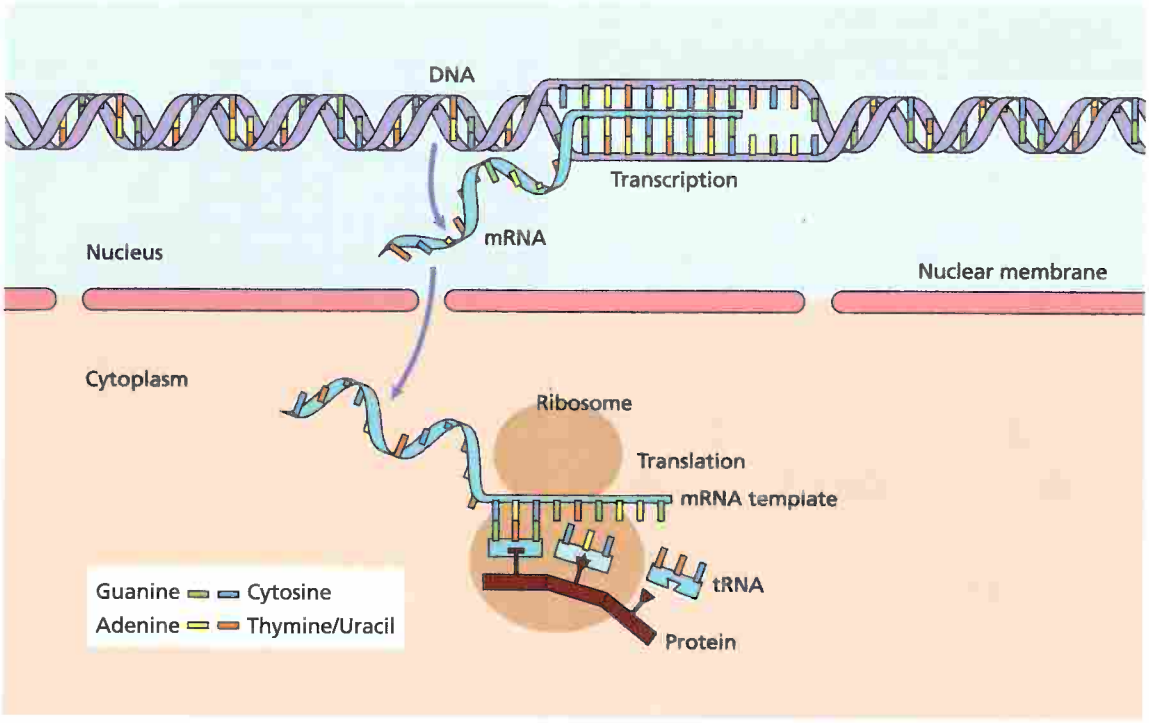

Figure 1 Genes are encoded in the DNA present in the nucleus of eukaryotic cells. RNA is the intermediary molecule that decodes the information in genes. This mRNA is exported from the nucleus to the cytoplasm where it is translated to allow synthesis of proteins. MicroRNAs (miRNAs) interfere with the synthesis of proteins encoded by mRNAs

From worms to all organisms

Research into miRNAs really took off in 2000 with the discovery of *let-7*, a regulator of development in *C. elegans*. Like *lin-4*, *let-7* is essential for larval worms to develop into adults. We now know that *let-7* is present in all animal and plant cells. In all cells it controls the cell cycle, cell differentiation and cell death and, through these processes, development. Different cell types in the body have different miRNAs and we now know that normal functioning of cells requires the activity of many different types of miRNA. miRNAs are as essential to life as proteins.

The average human cell contains over 1000 different miRNAs. These miRNAs can target and control over 60% of mRNAs that encode proteins. Similar to proteins, all miRNAs are encoded by genes. Some miRNA genes are clustered together in the DNA while others are present individually. The RNAs made from these miRNA genes can be ten times longer than the miRNA itself. These miRNA precursors are called pri-miRNAs and are highly structured pieces of nucleic acid present only in the nucleus of cells (see Figure 2). Pri-miRNAs need to be chopped up by biologically active proteins called enzymes to form mature miRNAs and are then transported to the cytoplasm to silence their target mRNAs.

How exactly do miRNAs silence mRNAs? miRNAs align through RNA–RNA base pairing (see Box 1) with the ends of mRNAs that encode

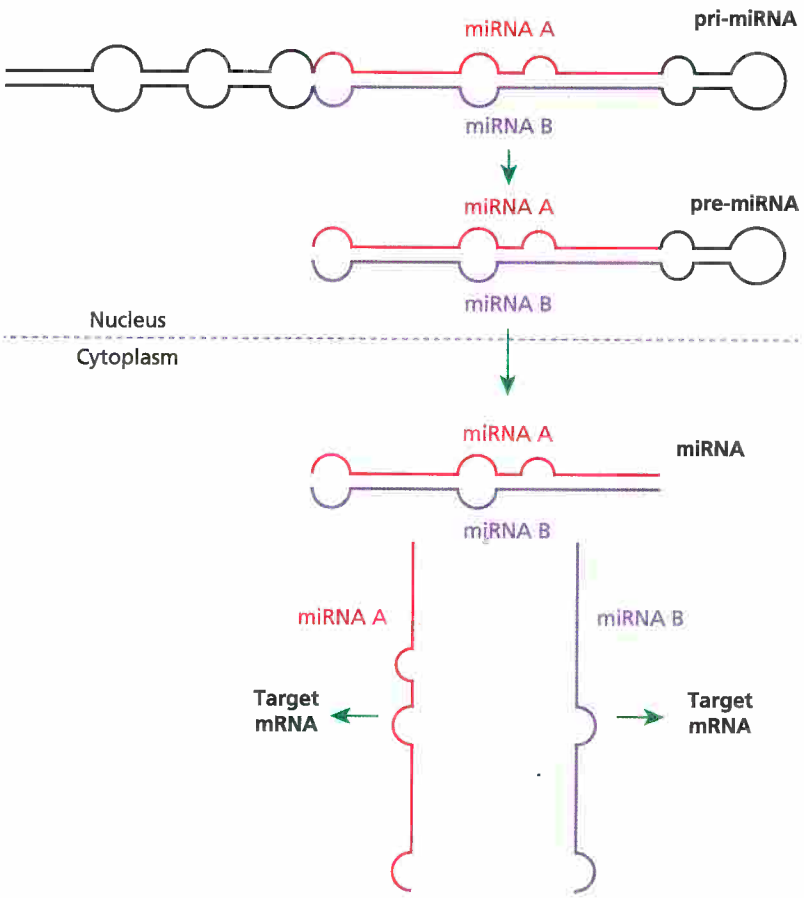

Figure 2 How miRNAs are made

Box 1 Base pairing

DNA and RNA are nucleic acid molecules made up of a phosphate–sugar backbone with four bases — adenine (A), cytosine (C), guanine (G) and thymine (T, DNA only) or uracil (U, RNA only). A can link with T or U and C can link with G. This linkage is known as base pairing and takes place via hydrogen bonds.

Because RNA is a flexible molecule it can bend and base pair with itself, for example as in the structure in Figure 1.1(a). miRNAs line up and link with their mRNA targets through base paring. If a C is lined up with an A or U, or an A is lined up with C or G (and so on), no base-pairing can occur so a bulge, such as that illustrated in Figure 1.1(b), is made in the RNA–RNA linkage.

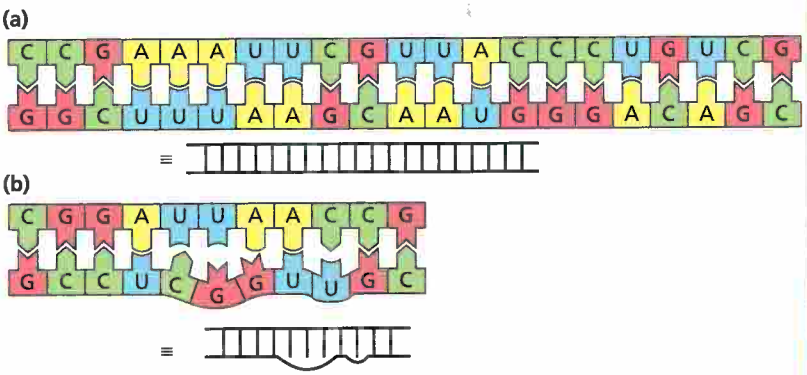

Figure 1.1 (a) A perfect 21 base pair RNA–RNA hybrid molecule. **(b)** A bulged 11 base pair RNA–RNA hybrid molecule

important proteins (see Figure 3). The RNA–RNA matches can be exact or can be a little mismatched, with bulges in the mRNA–miRNA linkages. Fully matched mRNAs–miRNAs are chewed up by special enzymes in the cytoplasm of the cell so the mRNAs can never be translated into proteins. More commonly, where there are mismatches or bulging, miRNA binding to mRNA seems to interfere with the formation of proteins encoded by the mRNA (see Figure 3).

The inhibition of protein synthesis from any one mRNA can be tightly controlled since a single mRNA is likely to be the target of multiple miRNAs. Although some miRNAs regulate individual mRNAs, others can be master regulators of several mRNAs. In many cases these target mRNAs encode proteins that have connecting functions in the cell. This coordination of mRNA targeting leads to efficient and stringent control of linked cellular pathways.

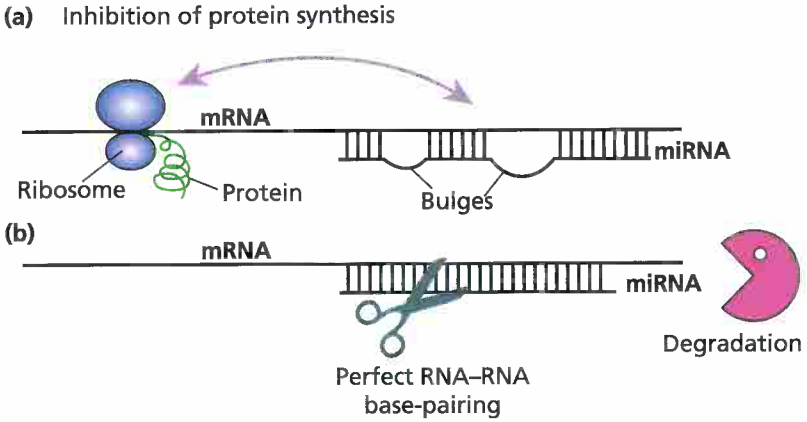

Figure 3 Two modes of action of miRNAs. **(a)** The miRNA binds to mRNA by base pairing but there are bulges because of some base mismatches. This situation results in blocking the production of the protein encoded by the mRNA. **(b)** The miRNA binds to mRNA and base pairing is complete. This means that the mRNA will attract special enzymes that chew up and get rid of mRNA

miRNAs in disease

Because of their essential roles in controlling the proteins that are made by cells, disruption of normal miRNA expression can lead to disease. We now know that malfunction of miRNAs is implicated in some liver diseases, diseases of the nervous system, cardiovascular disease, cancer and obesity. One of the most common inherited forms of mental retardation is called fragile X syndrome where affected individuals have X-chromosomes that break easily. This syndrome can be due to miRNA interaction with special mRNAs in affected individuals leading to loss of a protein that is essential for normal development of the nervous system.

It has recently been discovered that miRNAs are involved in cancer biology. Some miRNAs can suppress cancer formation and there are others that can promote cancer. For example, miR-21 is found at high levels in certain types of brain tumour cells. In experiments where miR-21 is removed from the cells, they stop growing and no longer behave like cancer cells. The first experiments that indicated the importance of miRNAs in cancer compared the miRNA present in normal cells with those present in cancer cells from the same tissue. There were clear differences in the miRNA present in the two cell types, i.e. cancer cells seem to possess specific miRNA makeup. Studies in mice showed that when cells expressed more of certain miRNAs, the mice got cancers.

There is now a database resource detailing the various miRNAs involved in different cancers. For example, levels of the *let-7* miRNA first discovered in worms are reduced by up to 80% in almost half of all lung tumours and very low *let-7* levels predict a poor outcome after treatment. This finding indicates that miRNAs have the potential to be good indicators of cancers, i.e. they could be used

Box 2 The wonderful world of RNA

There is a theory that the origins of life came from RNA molecules that were generated in the primordial soup. The more we learn about RNA the more this scenario seems likely, as most viruses have genetic material made of RNA rather than DNA. RNA can act as a physical scaffold and as a decoder in the interpretation of DNA into proteins. Certain RNAs can direct the functions of other molecules in cells and in the case of miRNAs, they can control what proteins a cell can make. In other words, they can establish and alter what a cell looks like and how it works. This means that RNA can accomplish most of the things that proteins are used for in cells. We now understand that we live in an RNA world!

Coloured chest X-ray of a patient showing metastatic (secondary) lung cancer (orange areas). Levels of *let-7* miRNA are usually low in lung tumours

to diagnose disease and predict the outcome of treatment. In future it is likely that miRNA profiling may be used to determine for individual patients what type of cancer they have, how aggressive it is and the best way of treating it. This personalised medicine approach, although costly at present, could result in significant improvements in treatment and economic savings in the long run for health services.

Therapeutic miRNAs

Certain miRNAs can control metastasis — the process by which cancer cells enter the bloodstream from the original tumour and are carried to other parts of the body to form a secondary tumour. Usually it is these secondary cancers that are difficult to treat and that become life threatening. miRNA-200 is often depleted during cancer metastasis due to genetic changes common to many cancer cells. This miRNA controls normal cell function. Therefore, if miRNA-200 could be reintroduced into metastatic cells, or if a way could be found to prevent it from being lost from these cells, then this could result in cancer cells becoming more normal.

If miRNAs are so important, then neutralising them might be a new means to combat a range of diseases. Recently some exciting reports in the scientific literature have shown that cells have miRNA 'sponges'. These are long RNA molecules that can soak up miRNAs by binding them through base pairing and preventing them from interacting with their normal mRNA targets. If it were possible to make synthetic miRNA sponges and introduce them into cells, we might be able to reverse the disease state caused by disease-associated miRNAs. This is potentially an exciting new dawn in development of drugs against disease.

Professor Sheila Graham works in the MRC-University of Glasgow Centre for Virus Research where she researches into RNA biology and viruses and cancer.

Key points

- miRNAs control animal and plant development.
- They are encoded by genes.
- Special enzymes are required to process long RNAs into miRNAs.
- miRNAs stop proteins being made from mRNAs.
- miRNAs are involved in many diseases including heart disease, neurological disorders and cancer.
- miRNAs could be the basis of therapy against disease.