the X-ray crystal structure of the ribosome, because it appears that the active site, where peptide units are linked, is constructed mostly or entirely from RNA; and that the RNA itself can catalyze the polymerization of peptide units. Hence, the ribosome itself may perhaps be regarded as a ribozyme – and a very ancient one too. The dual role of RNA both as a catalyst and also as an information carrier has supported much speculation that RNA was the first organic catalyst in the origin of life on Earth.

Certain plant and animal viruses contain RNA molecules that can cleave themselves. One particular example of such a naturally occurring, self-cleaving molecule is the *hammerhead ribozyme*, which may be isolated from small viruses that infect plants (known as 'viroids'); see Fig. A6(a). Several X-ray structures of the hammerhead ribozyme show that the molecule has a complex three-dimensional fold. The chemical mechanism of RNA-directed cleavage is thought to proceed by attack of a phosphate on the sugar 2′-OH group, as shown in Fig. A6(b).

Using the structural information from naturally-occuring ribozymes, scientists have designed artificial ribozymes that will recognize and cut specific messenger-RNA in cells. For instance, one such molecule uses a fragment of the hammerhead ribozyme which, like anti-sense RNA, recognizes a small section of the targeted messenger-RNA through complementary base-pairing, just as in Fig. A6(a). The messenger-RNA and ribozyme fragment co-fold to form an active ribozyme, which then cuts the messenger-RNA. For example, a hammerhead ribozyme has been designed to cleave the messenger-RNA that codes for a regulatory protein that is expressed aberrantly in breast cancer. When introduced into breast cancer cells in culture, that ribozyme restores a normal, non-cancerous lifetime to those cells. Other ribozymes have been used to target and destroy the messenger-RNA from HIV and hepatitis C virus; which stops these viruses from growing within the cell.

One advantage in using RNA to target the destruction of particular messenger-RNA is that it combines the catalytic potential of the molecule with the specificity provided by complementary base-pairing. Although the RNA ribozyme may have a rather complex, three-dimensional structure, procedures have been developed to isolate useful ribozymes by random selection and without the requirement to know the structure.

A complication of the ribozyme approach is that short RNA molecules are very sensitive to degradation by nucleases which are present in every cell. In order to get around that problem, some scientists have designed DNA-based ribozymes or 'DNAzymes', which can catalyze similar kinds of RNA-cleavage reactions. We

mentioned above that RNA ribozymes use the 2′ OH group to attack and cleave a sugar-phosphate backbone; yet that 2′ OH group is missing in DNA (see Fig. 10.3(a)). The reactive OH group is therefore provided by the target RNA itself, while the DNA helps to orient it

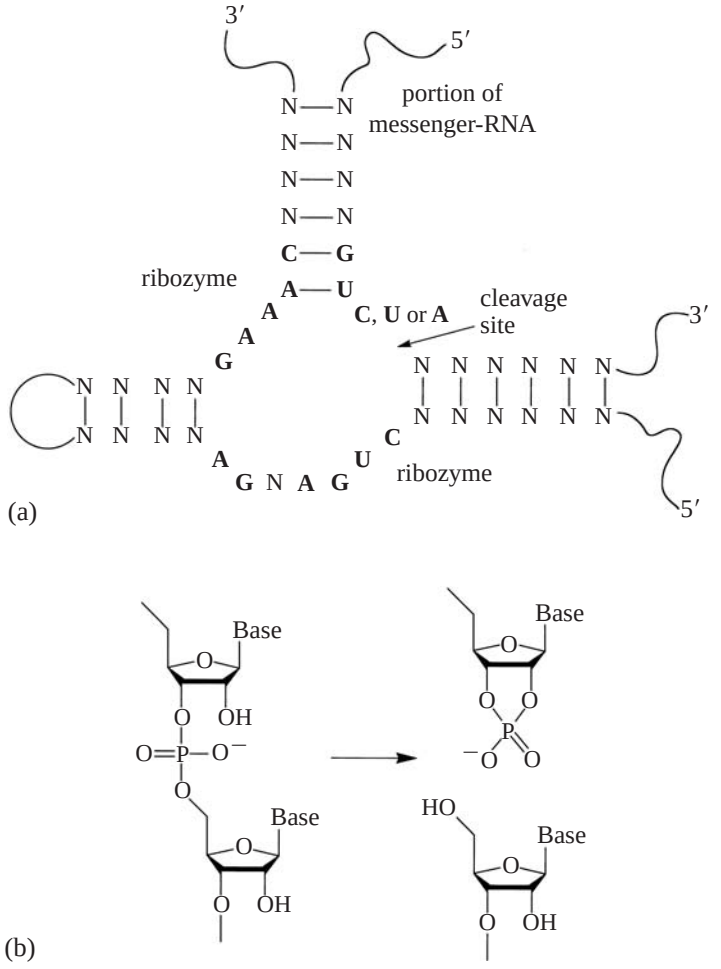

Figure A6 (a) Schematic diagram showing an RNA-cutting enzyme based on the naturally occurring 'hammerhead' RNA ribozyme from a plant viroid. An RNA molecule can be recognized by this ribozyme through complementary base-pairing, indicated by the lines connecting the bases; and it will then be cleaved at a specific point. The specific residues required for maintaining the appropriate structure for catalysis are in bold type, and all other bases are indicated by N. The RNA to be cut will co-fold with a ribozyme fragment in order to generate the cutting activity. (b) Chemical changes resulting from cutting by the ribozyme. The OH group on the 2′ position of the sugar is positioned by the hammerhead structure to initiate an attack on the phosphate. This breaks the linkage between the adjacent bases, leaving a cyclic 2′, 3′ product on the base with the reactive 2′ OH, and an OH group on the other fragment.

for the cleavage reaction. DNAzymes have been developed that catalyze other processes, such as incorporating metals into heme rings. Most DNAzymes require cofactors, such as metals or amino acids, that assist in the catalysis and may provide the catalytic groups.

si-RNA

In the cells of plants and animals, certain double-stranded RNA molecules may repress the translation of specific genes in a controlled manner. There, an evolutionarily conserved process silences any particular messenger-RNA which contains the same sequence as that of the short double-stranded RNA. First, a ribonuclease called 'Dicer' (e.g. in plants, flies and worms) cuts long double-helical RNA into many small pieces of size 21 to 23 base-pairs, with two-base extended 3′ ends. These are called 'small interfering RNA', or si-RNA. Those small fragments then bind to a large ribonuclease complex called 'RISC' (for 'RNA induced silencing complex'), which is 'guided' by the small interfering RNA to destroy any messenger-RNA that contains an identical sequence (Fig. A7).

Small interfering RNA may act also through a polymerase-catalyzed amplification in worms or plants, where an RNA-directed polymerase uses the starting RNA segment as a primer, to make more interfering RNA along distant segments of the same message. Thus, even a few RNA molecules can be amplified in the cell, and can thereby have a very potent effect on gene expression.

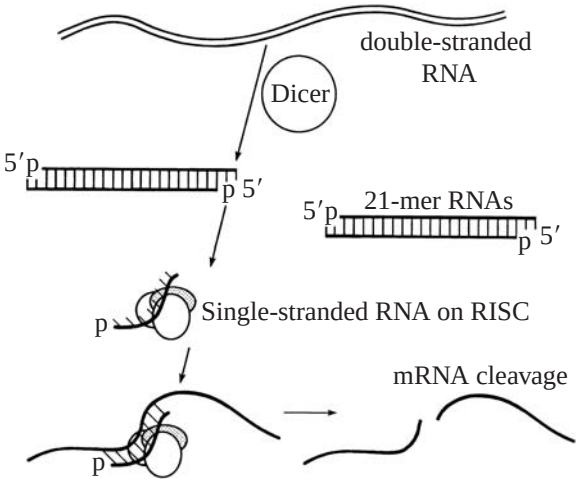

Figure A7 A probable mechanism of action for small interfering RNA. The double-stranded complex that is formed between short si-RNA and long messenger-RNA can be cleaved efficiently by a cellular ribonuclease assembly called 'RISC'.

But why should any cell have developed such a potentially hazardous system of RNA degradation? The si-RNA machinery probably evolved first as a mechanism of defence against RNA viruses, which may adopt a double-stranded form transiently during RNA-to-RNA replication. Later in evolution it may have became involved in regulating DNA methylation (see Chaper 11); and now it has proven very powerful at silencing gene expression in cells by the specific degradation of certain messenger-RNAs.

To activate the RNA interference system, one must first introduce long double-stranded RNA into a cell, one strand of which is complementary to the chosen messenger-RNA, for cells which contain Dicer; or else introduce small pieces of double-stranded RNA, 21 to 23 base-pairs in length with two-base 3′ overhangs, for cells which do not contain Dicer.

One important application of si-RNA so far has been to study gene usage in animal cellular development, for example in the worm *Caenorhabditis elegans*, where much progress has already been made. Scientists have found an easy method to inactivate any specific gene within that worm, by feeding it bacteria which make an RNA that targets the desired worm messenger-RNA. So, at least in the worm, gene inactivation by si-RNA works well. Soon, perhaps, anti-sense, ribozyme and si-RNA may become important as well for transgenic animals, or even for modern medicine, to silence defective single-copy genes in humans.

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Answers to Selected Exercises

Note: most numerical values are given to more significant figures than are warranted by the data, in order to provide a better check on the arithmetic.

1.1 **a** Length of total DNA/diameter of cell = 200 000.
b Volume of total DNA/volume of cell = 0.01.
c Diameter of typical compact DNA ball = 6400 Å = 0.64 μm.
Length of typical metaphase chromosome = 4.3 μm.

1.2 **a** Ala, Lys, Gln, Leu, Ile, Gln, Gly.
b Pro, Ser, Asn, Ser, Phe, Lys.
Gln, Ala, Thr, His, Ser, Arg.

1.3 **a** Ala, Lys, Gln, Arg, His, Ser, Arg.
b Ala, Lys, Gln, Ser, Phe, Lys.

1.4 **a** Met, Ser, His, Gly, Thr, (Stop).
b Met, Val, Ile, Arg, Asn, Ser, (Stop).

2.1 **a** 29°, 12.4 bp/turn.
b 9.0 bp/turn.
c 9.9 bp/turn.

2.2 **a** 330 Å, 10.0 turns.
b 363 Å, 9.3 turns.
c 495 Å, 6.4 turns.

2.5 **a** 2.
b 21, 3.

3.1 Right-handed or clockwise.

3.6 'A': 4.7 Å, 22°, 2.5 Å
'B': 0 Å, 0°, 3.3 Å
'C': −2.2 Å, −9°, 3.1 Å.

4.2 **b** There are 3 AT-rich and 2 GC-rich regions.
c There are 8 pyrimidine-purine steps.
d GGCCC is the strongest region, and TATATA the weakest.

4.3 All bases except GCGC and CCGG at the two ends can make a cruciform about the central loop CTAG.

4.4 **a** 18, 10.8 m, 1.72 m.
b 0.58 rad/m, 1.72 m.

4.5 **a** 30°, 0°, **b** 15°, −15°, **c** 9.3°, 0°, **d** 6°, −6°.

4.6 **a** 26.2°, **b** −26.2°, **c** 26.2°, **d** 30°, **e** 30°.

4.7 To adenine.

5.1 **a** −45°, 14 bp, −90 bp, 127 bp.
b 45°, 14 bp, 90 bp, 127 bp.
c 6°, 28 bp, 18 bp, 179 bp.
d 84°, 3 bp, 178 bp, 179 bp.
Helix **d** has the smallest diameter, and helix **c** the largest. Helix **a** is left-handed, while helices **b**, **c**, **d** are right-handed.

5.2 **a** 'A': 30.7°, +11.2°
'B': 36.0°, 0.0°
'C': 39.4°, −7.0°.
b 34.0°.

5.3 **b** first sum = 24.9°, second sum = 8.1°, k = 26.2°, phase = 72°, maximum roll R is at step 2.

5.4 **a** 0.86°/bp, step 6.
b −1.7°/bp.
c 45 Å, 189 bp.

5.5 **a** 1.07°/bp, step 7.
b −1.5°/bp.
c 60 Å, 195 bp.

5.6 **a** 1.05°/bp, step 7.5.
b 1.7°/bp.
c 50 Å, 180 bp.
A_4N_6 and A_6N_4 are left-handed, while A_6N_5 is right-handed.

6.1 **a** Once, approximately.
b Once, approximately, depending on the kind of cord used.

6.4 **a** 2 turns, left-handed.
b +1 turn, so that +1 −2 = −1. To reduce the supercoiling or tangling of DNA cell division.

6.5 Tw = 0, −0.50, −0.87, −1.00 turns
Wr = −1.00, −0.50, −0.13, 0 turns.

7.1 **a** 214 ± 13, 429 ± 15, 644 ± 20, 855 ± 21.
b 214.5 base-pairs.

7.2 **a** DNA occupies 53% of total volume.
b 76 bp/hoop.

7.3 **a** **a** 291 Å. **b** 87 Å. **c** 100 Å.
b **a** 300 Å. **b** 100 Å. **c** 50 Å.

7.4 **a** No.
b No, they would join readily.

7.5 Nucleosome, 300 Å fiber, loops, metaphase coiling.

9.1 **a** Phosphorus, oxygen, nitrogen, carbon, hydrogen.
b The phosphate PO_4 at lower-center left.
c Because hydrogen scatters X-rays so weakly (36 times less strongly than carbon).

9.2 **a** 5. **b** 6. **c** 10.

9.3 **a** Four kinds of fragment, of size 100, 200, 250, 450 bp.
b 100 will be the fastest, and 450 the slowest.

9.4 **a** 125, **b** 7.5×10^6, i.e. 7.5 million.

9.5 For A_6N_4, pitch $p = 526$ Å, volume of circumscribing cylinder $= 8.01 \times 10^6$ Å^3, and ratio of volume for curved *versus* straight DNA = 39.6. For other sequences from Table 5.1, the volume ratios are $A_6N_2 = 1.4$, $A_6N_3 = 2.7$, $A_6N_5 = 29.5$, $A_6N_6 = 3.7$ and $A_6N_7 = 1.9$. Hence A_6N_4 and A_6N_5 go most slowly through a gel, while the speed of the other sequences is not much different from that of straight DNA.

9.6 Wr = 0 for slowest; and since Tw = −12, then Lk = −12 for slowest (check: this is within the given range). Fastest has the value of Wr which differs most from zero; and since Tw = −12, the range of Wr is from +12 to −8 for Lk = 0 to −20. Therefore Wr = +12, Lk = 0 is the fastest.