

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.

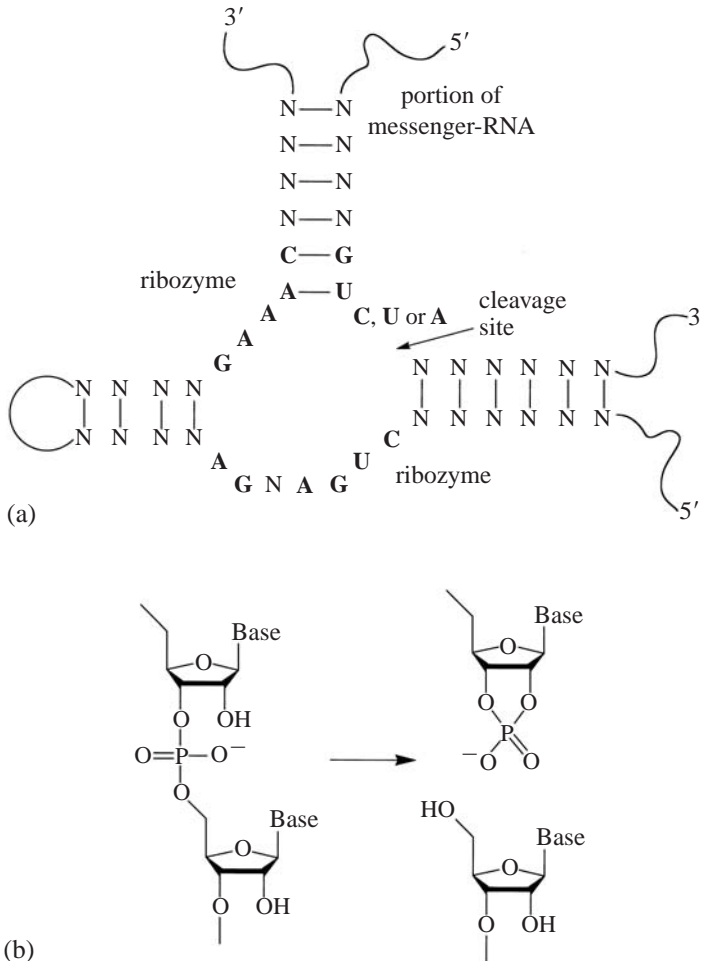
Certain plant and animal viruses contain RNA molecules that can cleave themselves. One particular example of such a naturally occurring, self-cleaving molecule is the *hammerhead ribozyme*, which may be isolated from small viruses that infect plants (known as 'viroids'); see Fig. A6(a). Several X-ray 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-occurring 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



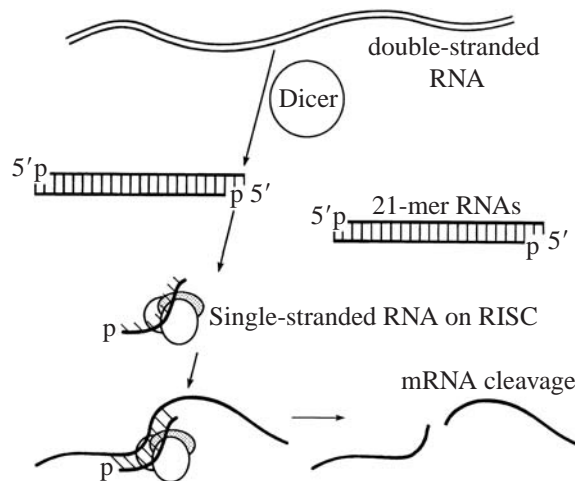
**Figure A6** (a) Schematic diagram showing an RNA-cutting enzyme based on the naturally occurring 'hammerhead' RNA ribozyme from a plant viroid. An RNA molecule can be recognized by this ribozyme through complementary base-pairing, indicated by the lines connecting the bases; and it will then be cleaved 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 become involved in regulating DNA methylation (see Chapter 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.

## Further Reading and Bibliography

### **Antisense oligomers**

- Braasch, D.A. and Corey, D.R. (2002) Novel antisense and peptide nucleic acid strategies for controlling gene expression. *Biochemistry* **41**, 4504–10. A summary of chemically synthesized anti-sense oligomers which are currently available; their mechanisms of action which may reduce gene expression, as well as a summary of current clinical trials.
- Sazani, P., Gemignani, F., Kang, S.-H., Maier, M.A. *et al.* (2002) Systemically delivered antisense oligomers upregulate gene expression in mouse tissues. *Nature Biotechnology* **20**, 1228–33. Cationic PNA-lysine or 2'-O-methoxyethyl-phosphorothioate anti-sense oligomers work well to influence messenger-RNA splicing in mouse.

### **Peptide nucleic acid**

- Borgatti, M., Lampronti, I., Romanelli, A., Pedone, C. *et al.* (2003) Transcription-factor decoy molecules based on a PNA-DNA chimera

- mimicking Sp1 sites. *Journal of Biological Chemistry* **278**, 7500–9. Double-helical mixtures of DNA and PNA can bind to transcription factors such as Sp1, and alter their *in vivo* activities.
- Cartegni, L. and Krainer, A.R. (2003) Correction of disease-associated exon skipping by synthetic exon-specific activators. *Nature Structural Biology* **10**, 120–5. Covalent coupling of PNA to a splicing activator can restore splicing.
- Dias, N., Senamaud-Beaufort, C., le Forestier, E., Auvin, C. *et al.* (2002) RNA hairpin invasion and ribosome elongation arrest by mixed-base PNA oligomer. *Journal of Molecular Biology* **320**, 489–501. Certain PNA molecules which form triple helices can block translation of messenger-RNA at the ribosome.
- Eriksson, M., Nielsen, P., and Good, L. (2002) Cell permeabilization and uptake of antisense peptide-PNA into *Escherichia coli*. *Journal of Biological Chemistry* **277**, 7144–7. The covalent coupling of PNA and a membrane-permeable peptide enables them to enter a bacterial cell.
- Kaushik, N., Basu, A., and Pandey, V.N. (2002) Inhibition of HIV-1 replication by anti-trans-activation responsive polyamide nucleotide analog. *Antiviral Research* **56**, 13–27. Certain PNA molecules can block HIV replication in cell culture.

### ***Ribozymes or DNAzymes and ribosomes***

- Doudna, J.A. and Cech, T.R. (2002) The chemical repertoire of natural ribozymes. *Nature* **418**, 222–8. A survey of self-cleaving RNA structures and their possible functions.
- Feldman, A.R. and Sen, D. (2001) A new and efficient DNA enzyme for the sequence-specific cleavage of RNA. *Journal of Molecular Biology* **313**, 283–94. The use of DNAzymes as well as ribozymes.
- Guerrier-Takada, C., Gardiner, K., Marsh, T., Pace, N., and Altman, S. (1983) The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme. *Cell* **35**, 849–57. This work, and the study by Kruger *et al.*, showed for the first time that RNA itself can have a catalytic function.
- Haseloff, J. and Gerlach, W.L. (1988) Simple RNA enzymes with new and highly specific endoribonuclease activities. *Nature* **334**, 585–91. Discovery of the hammerhead ribozyme from a plant viroid, used to cut a messenger-RNA.
- Nissen, P., Hansen, J., Ban, N., Moore, P.B., and Steitz, T.A. (2000) The structural basis of ribosome activity in peptide bond synthesis. *Science* **289**, 920–30. The crystal structure of the large subunit of the ribosome, and the hypothesis that peptide-bond formation is catalyzed by the RNA itself.
- Santoro, S.W. and Joyce, G.F. (1997) A general-purpose RNA-cleaving DNA enzyme. *Proceedings of the National Academy of Sciences, USA* **94**, 4262–6. Design of a general and efficient DNA enzyme that can cleave HIV-1 genomic RNA in the test tube.
- Scott, W.G., Finch, J.T., and Klug, A. (1995) The crystal structure of an all-RNA hammerhead ribozyme – a proposed mechanism for RNA catalytic cleavage. *Cell* **81**, 991–1002.

- Sullenger, B.A. and Gilboa, E. (2002) Emerging clinical applications of RNA. *Nature* **418**, 252–8. The use of ribozymes for clinical purposes such as treatment of HIV.
- Takagi, Y., Suyama, E., Kawasaki, H., Miyagishi, M., and Taira, K. (2002) Group II introns and mRNA splicing. *Biochemical Society Transactions* **30**, 1145–9. The selection of variant ribozymes from randomized libraries.

## si-RNA

- Elbashir, S.M., Lendeckel, W., and Tuschl, T. (2001) RNA interference is mediated by 21- and 22-nucleotide RNAs. *Genes and Development* **15**, 188–200. How synthetic small RNAs activate the RNA interference process in the cell.
- Gupta, B.P., Wang, M., and Sternberg, P.W. (2003) The *C. elegans* LIM homeobox gene *lin-11* specifies multiple cell fates during vulval development. *Development* **130**, 2589–601. An example of how RNAi is used to study specific events in the development of the worm.
- Hannon, G.J. (2002) RNA interference. *Nature* **418**, 244–51. An excellent summary of RNA-mediated gene silencing in plants, worms, flies and human cells, and its short-term therapeutic applications.
- Hsu, A.L., Murphy, C.T., and Kenyon, C. (2003) Regulation of aging and age-related disease by DAF-16 and heat shock factor. *Science* **300**, 1142–5. Studies of specific genes which influence aging in a worm, often by use of si-RNA.
- Miller, V.M., Xia, H., Marrs, G.L., Gouvion, C.M. *et al.* (2003) Allele-specific silencing of dominant disease genes. *Proceedings of the National Academy of Sciences, USA* **100**, 7195–200. Mutant protein which causes SCA-3 neural disease in humans may be destroyed, by using si-RNA targeted against messenger-RNA made from one chromosome but not the other.
- Miyagishi, M., Hayashi, M., and Taira, K. (2003) Comparison of the suppressive effects of antisense oligonucleotides and si-RNAs directed against the same targets in mammalian cells. *Antisense and Nucleic Acid Drug Development* **13**, 1–7. Si-RNA works 100 times more efficiently per molecular copy than anti-sense, and far more than a ribozyme, at reducing gene expression in a model system.
- Reinhart, B.J. and Bartel, D.P. (2002) Small RNAs correspond to centromere heterochromatic repeats. *Science* **297**, 1831. ‘Silencing’ of centromeric heterochromatin in yeast by small RNAs made by the centromeric repeat regions. Also describes possible links between methylation state and RNA interference, showing that the RNA interference mechanism can contribute to epigenetic effects as described in Chapter 11.
- Tang, G., Reinhart, B.J., Bartel, D.P., and Zamore, P.D. (2003) A biochemical framework for RNA silencing in plants. *Genes and Development* **17**, 49–63. Si-RNA acts in plants as well as worms or mammals for gene silencing.
- Tuschl, T. (2002) Expanding small RNA interference. *Nature Biotechnology* **20**, 446–8. Methods for expression of si-RNA from polymerase III promoters *in vivo*.

Yokota, T., Sakamoto, N., Enomoto, N., Tanabe, Y., Miyagishi, M., Maekawa, S., Yi, L., Kurosaki, M., Taira, K., Watanabe, M., and Mizusawa, H. (2003) Inhibition of intracellular hepatitis C virus replication by synthetic and vector-derived small interfering RNAs. *EMBO Reports* **4**, 602–8. Demonstrates the feasibility of using RNA interference to inhibit viral replication.

# 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 =  $6400 \text{ \AA} = 0.64 \text{ }\mu\text{m}$ .  
Length of typical metaphase chromosome =  $4.3 \text{ }\mu\text{m}$ .
- 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  $29^\circ$ , 12.4 bp/turn.  
b 9.0 bp/turn.  
c 9.9 bp/turn.
- 2.2 a  $330 \text{ \AA}$ , 10.0 turns.  
b  $363 \text{ \AA}$ , 9.3 turns.  
c  $495 \text{ \AA}$ , 6.4 turns.
- 2.5 a 2.  
b 21, 3.
- 3.1 Right-handed or clockwise.
- 3.6 'A':  $4.7 \text{ \AA}$ ,  $22^\circ$ ,  $2.5 \text{ \AA}$   
'B':  $0 \text{ \AA}$ ,  $0^\circ$ ,  $3.3 \text{ \AA}$   
'C':  $-2.2 \text{ \AA}$ ,  $-9^\circ$ ,  $3.1 \text{ \AA}$ .
- 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**  $30^\circ$ ,  $0^\circ$ , **b**  $15^\circ$ ,  $-15^\circ$ , **c**  $9.3^\circ$ ,  $0^\circ$ , **d**  $6^\circ$ ,  $-6^\circ$ .

4.6 **a**  $26.2^\circ$ , **b**  $-26.2^\circ$ , **c**  $26.2^\circ$ , **d**  $30^\circ$ , **e**  $30^\circ$ .

4.7 To adenine.

5.1 **a**  $-45^\circ$ , 14 bp,  $-90^\circ$ , 127 bp.

**b**  $45^\circ$ , 14 bp,  $90^\circ$ , 127 bp.

**c**  $6^\circ$ , 28 bp, 18 bp, 179 bp.

**d**  $84^\circ$ , 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':  $30.7^\circ$ ,  $+11.2^\circ$

'B':  $36.0^\circ$ ,  $0.0^\circ$

'C':  $39.4^\circ$ ,  $-7.0^\circ$ .

**b**  $34.0^\circ$ .

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

5.4 **a**  $0.86^\circ/\text{bp}$ , step 6.

**b**  $-1.7^\circ/\text{bp}$ .

**c** 45 Å, 189 bp.

5.5 **a**  $1.07^\circ/\text{bp}$ , step 7.

**b**  $-1.5^\circ/\text{bp}$ .

**c** 60 Å, 195 bp.

5.6 **a**  $1.05^\circ/\text{bp}$ , step 7.5.

**b**  $1.7^\circ/\text{bp}$ .

**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**  $214 \pm 13$ ,  $429 \pm 15$ ,  $644 \pm 20$ ,  $855 \pm 21$ .

**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  $\text{PO}_4$  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**  $7.5 \times 10^6$ , i.e. 7.5 million.
- 9.5 For  $\text{A}_6\text{N}_4$ , pitch  $p = 526 \text{ Å}$ , volume of circumscribing cylinder  $= 8.01 \times 10^6 \text{ Å}^3$ , and ratio of volume for curved *versus* straight DNA  $= 39.6$ . For other sequences from Table 5.1, the volume ratios are  $\text{A}_6\text{N}_2 = 1.4$ ,  $\text{A}_6\text{N}_3 = 2.7$ ,  $\text{A}_6\text{N}_5 = 29.5$ ,  $\text{A}_6\text{N}_6 = 3.7$  and  $\text{A}_6\text{N}_7 = 1.9$ . Hence  $\text{A}_6\text{N}_4$  and  $\text{A}_6\text{N}_5$  go most slowly through a gel, while the speed of the other sequences is not much different from that of straight DNA.
- 9.6  $\text{Wr} = 0$  for slowest; and since  $\text{Tw} = -12$ , then  $\text{Lk} = -12$  for slowest (check: this is within the given range). Fastest has the value of  $\text{Wr}$  which differs most from zero; and since  $\text{Tw} = -12$ , the range of  $\text{Wr}$  is from  $+12$  to  $-8$  for  $\text{Lk} = 0$  to  $-20$ . Therefore  $\text{Wr} = +12$ ,  $\text{Lk} = 0$  is the fastest.