

Further Reading

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Exercises

7.1 A piece of double-helical DNA, 3010 base-pairs long, is bound in a test tube to a series of histone octamers in the manner of Figs 7.1, 7.2 and 7.3. Then an enzyme like the one used by Hewish and Burgoyne (1973) is added to a sample. Fine-mapping experiments show that the enzyme cuts only at certain locations along the length of the DNA molecule, which are known to be the empty spaces or ‘linkers’ between histone octamers. These locations, measured in terms of the distance from one end of the 3010-bp DNA, were found by experiment to be (in units of base-pairs): 220, 430, 670, 870, 1090, 1320, 1520, 1740, 1930, 2140, 2350, 2580, 2790.

- a Make a table of all possible DNA fragment lengths, for fragments containing either 1, 2, 3, or 4 histone octamers. This table should have four columns. Column 1 should list all fragment lengths of size $220 - 0 = 220$, $430 - 220 = 210$, etc. base-pairs, and should contain 14 numbers. Column 2 should list fragment lengths of size $430 - 0 = 430$, $670 - 220 = 450$, etc., and should contain 13 numbers. Column 3 should list fragments such as $670 - 0 = 670$, $870 - 220 = 650$, and contain 12 numbers. Finally, column 4 should list sizes of $870 - 0 = 870$, $1090 - 220 = 870$, etc., and contain 11 numbers.

What is the mean fragment size in each of columns 1, 2, 3, 4, and its standard deviation?

- b What is the mean spacing of histone octamers in this particular sample? Divide each mean fragment size by the column number 1, 2, 3, or 4 to get an optimal value.

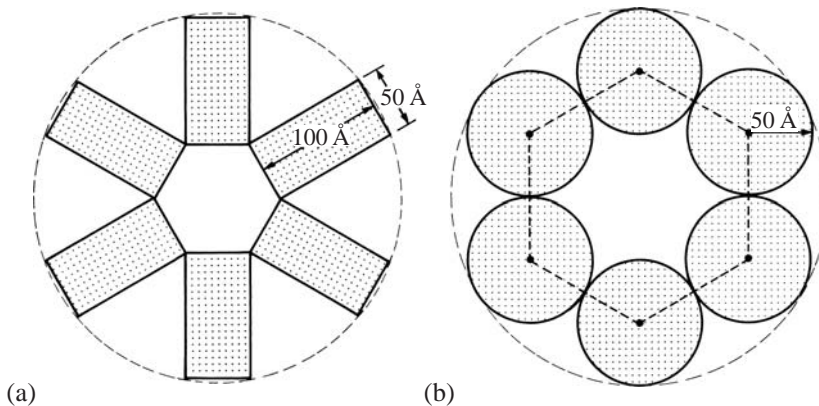
7.2 The model of the histone octamer and its associated DNA, that is shown in Fig. 7.2, may be represented crudely as a protein cylinder of diameter 60 \AA and height 50 \AA , around which are wrapped two complete turns of DNA having a diameter of 20 \AA .

- Calculate the proportion of the total volume which is occupied by the DNA. For this rough calculation you may treat the DNA as two separate hoops, each with an inner diameter of 60 \AA . Recall that the volume of a hoop is equal to the product of its cross-sectional area and its mid-circumference.
- Calculate the number of base-pairs in each hoop, assuming that the DNA has a length of 3.3 \AA per base-pair.

7.3 The packing of nucleosomes into a usual model for the '300 \AA fiber', as shown in Fig. 7.3, may be represented approximately by diagram (a), below. Here, to make the calculations especially simple, we have drawn the nucleosomes as closed rings of six, rather than as helical spirals with six nucleosomes per turn.

By considering each nucleosome as a cylinder of radius 50 \AA and length 50 \AA (for the protein plus DNA: see Exercise 7.2), estimate **a** the outer diameter of each ring, **b** the diameter of the inner hole, and **c** the height of the 300 \AA fiber per ring of six nucleosomes.

A second possible packing scheme, also expressed in terms of closed rings, is shown in diagram (b) below. The nucleosomes are cylinders, just as before, but now their flat faces lie perpendicular to the axis of the 300 \AA fiber, instead of lying parallel to this axis, as in diagram (a).



Compute the dimensions **a–c** also for model (b); and compare them with the corresponding figures for model (a). (See Widom, J. and Klug, A. (1985) *Cell* 43, 207–13, for evidence about key dimensions, and in particular that the axial spacing **c** is $\approx 110 \text{ \AA}$.)

7.4 The telomeres of human chromosomes are made from a long, multiple repeat of the sequence (5') TTAGGG (3'), as stated in the caption of Fig. 7.6. Often, such a repeat projects beyond the end of the double-helical DNA at each end of the chromosome, to leave a short, single strand of 6 unpaired bases as shown below:

(5') ...TTAGGGTTAGGGTTAGGG (3')
 (3') ...AATCCCAATCCC (5')

Such an arrangement is known as a 'sticky end' of DNA. There are known to be many enzymes in the cell that can join or 'fuse' two such single-stranded ends of DNA molecules to one another, if they detect sufficient Watson–Crick base-pairing.

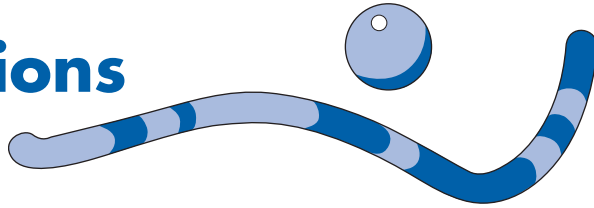
- a** Could two identical (5') TTAGGG (3') ends like the one shown above (with the second one found by rotating the first through 180° in the plane of the diagram) be joined by such an enzyme?
- b** Suppose that human telomeres were made not from repeats of TTAGGG, but from repeats of some other sequence such as TAGCTA or CGATCG. Would such hypothetical telomeres prevent the ends of chromosomes from joining to one another by Watson–Crick pairing? (See Ijdo, J.W., Baldini, A., Ward, D.C., Reeders, S.T., and Wells, R.A. (1991) *Proceedings of the National Academy of Sciences, USA* **88**, 9051–5, for a rare instance of telomere fusion.)

7.5 It is often possible to prepare, in the laboratory, a 'soup' or extract of proteins from a living cell which is able to synthesize some particular RNA molecule when a DNA 'template' molecule, having a specific sequence, is added to it.

If you study the process of transcription solely in this cell extract, on 'naked', histone-free DNA, what important features of the chromosome structure – features that could affect the activity of a DNA template in a living cell – would be excluded from your study? By scanning the diagrams of this chapter, identify at least four different structures of DNA within a chromosome that might influence gene activity in this way. (See Felsenfeld, G. (1992) *Nature* **355**, 219–24, for a survey of such concerns.)

CHAPTER 8

Specific DNA–Protein Interactions



In the previous chapter we explained how the vast majority of chromosomal DNA is associated with packaging proteins, such as histones. Those abundant proteins bind to most parts of the lengthy chromosomal DNA with only a slight preference for base sequence; and in organisms whose cells have nuclei, they serve mainly to compact the long DNA by a factor of about 10 000, into the minute volume of a cell nucleus. Chromosome compaction can control the access of RNA polymerase enzyme to the start site of genes (i.e., the ‘promoter’ sequences) and thereby affect the transcription of DNA into RNA. However, the study of packaging proteins alone cannot teach us how specific genes might be controlled, during the growth or development of cells into a mature organism, or during cellular response to environmental stimuli, or during the routine ‘housekeeping’ activities of fully developed cells.

For that reason, scientists have also studied the many less abundant DNA-binding proteins that bind specifically just to a small part of the long chromosomal DNA, in the proximity of the start sites for genes, or in other important locations. Some of these proteins can bind to DNA with high specificity for the base sequence, and can thereby carry out many specific biological tasks, such as control of transcription, repair of damaged DNA or unwinding of DNA. A few of these tasks are shown in Fig. 8.1.

The first DNA-binding proteins studied by scientists were regulatory proteins from bacteria, where they act to control perhaps the simplest genetic systems found in Nature. Many of these bacterial proteins act as ‘*repressors*’ of gene activity (see the upper part of the picture) if they bind tightly to a base-sequence of DNA which overlaps the ‘promoter’ sequence, where an RNA polymerase enzyme

can also bind. They can thereby prevent the binding of RNA polymerase to a particular promoter, through direct competition for the same local segment of DNA. In general, such repressor proteins reduce the rate at which RNA is made from a promoter; and indeed such repression of RNA synthesis may be specific to just one or a few genes in an entire organism, if the repressor binds to only one or a few sites on an entire chromosome.

In bacteria, repressor proteins play an important role in reducing local rates of transcription; but in plants, animals and other organisms whose cells have nuclei – known collectively as eukaryotes¹ – the chromosome structure itself tends to repress transcription. Indeed, in nucleated organisms it is the activation of genes that seems to be the more important aspect of gene regulation. That process is managed by ‘activators’ of transcription (see the middle part of Fig. 8.1) that bind specifically to DNA in the general vicinity of a binding site for RNA polymerase. The activator protein may then increase the rate at which RNA is made, by directly assisting the RNA polymerase enzyme and its auxiliary proteins to bind at the promoter sequence, through a network of protein-to-protein

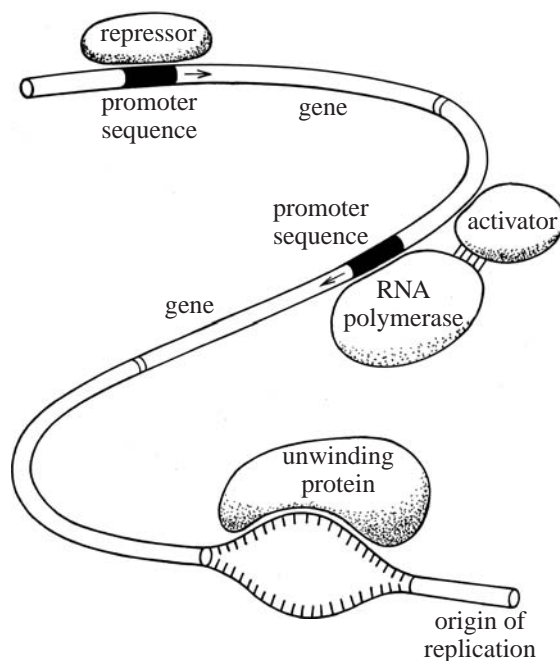


Figure 8.1 Proteins of various kinds may bind specifically to different DNA sequences, so as to carry out important biological tasks such as the repression or activation of individual genes, or unwinding the double helix in specific sites for copying or replication. A highly schematic picture, showing some of the DNA–protein interactions described in this chapter.

contacts; or else indirectly by helping to ‘recruit’ enzymes that can chemically modify the chromatin (see Chapter 7). For instance, certain transcription activators may direct histone acetylases to the general region of a specific gene. The resulting modification of histones may cause the chromatin to decompact near that promoter, and thereby make it more accessible to RNA polymerase and its auxiliary proteins.

‘DNA looping’ may represent a somewhat more complex example of how genes are regulated in three dimensions, and not just in one or two, by some linear or planar arrangement of DNA binding sites. In the latter case, two or more repressor or activator proteins may bind to the same piece of DNA, and then join together to create a small loop or coil, which can affect gene activity very strongly (either positively or negatively) on account of its stable structure.

We have mentioned so far repressors and activators and loops, but there are also many other kinds of regulatory protein that interact with the DNA, and so play important roles in the cell. Thus, a fourth kind of DNA-binding protein (see the lower part of Fig. 8.1) may help to unwind the DNA double helix near an ‘origin of replication’, where the duplication of old DNA into new DNA starts during every cell division. After such unwinding by these special kinds of protein, a DNA polymerase enzyme can read the bases on each separate strand by means of Watson–Crick pairing and then copy them; and so can make two new DNA double-helices from the two old strands. Also, proteins which bind to DNA may carry out many different biological tasks not shown here, such as the supercoiling of DNA, or the exchanging of DNA segments in ‘recombination’. The cell also has elaborate assemblies of enzymes that recognize and repair DNA damage, which is a crucial aspect of cell viability.

In practice, all of the pictures shown in Fig. 8.1 are *greatly oversimplified*, since they omit so much important detail. For example, each specific interaction between protein and DNA must be highly complex from a chemical point of view, in order to distinguish one base sequence from others over the length of an entire chromosome. Also, each protein may be regulated in its ability to bind to specific sequences on the DNA, by a variety of factors including: (a) interaction with other proteins nearby; (b) any modification of the DNA such as base methylation (see Chapter 11); or (c) by binding to small molecules such as sugars and amino acids present in the cellular medium.

How can anyone hope to learn anything useful from such a cacophony of information, without making a big dictionary that would run to thousands of pages, and searching through the facts one at a time? The only hope for greater understanding seems to be