light and the electron microscope. In other words, the 300 Å fibers seem to be disposed in the nucleus rather loosely, like a bunch of spaghetti in a bowl, as shown schematically in Fig. 1.2. It is only when a cell is on the point of dividing that these fibers condense or fold into structures that are sufficiently compact to be seen by use of a light or electron microscope. Such highly compact structures are known as 'metaphase'[1] chromosomes: some pictures of them were shown in Fig. 1.3. Clearly, the 300 Å fibers within metaphase chromosomes must be packed rather densely around each other into some sort of regular array, although we do not understand at present how this is accomplished.

Yet it seems likely that the 300 Å fibers in their dispersed or 'interphase'[1] state are not organized quite so loosely or randomly as spaghetti in a bowl. They must eventually undergo compaction when a cell divides at metaphase, and it is hard to see how they could do this if they were dispersed entirely at random during the intervening periods. Therefore, we should look for an additional level of structure between that of the 300 Å fiber and the condensed, metaphase chromosome.

The strongest evidence for such an intermediate level of organisation comes from the strange 'polytene' chromosomes which are found in the salivary gland of the fruit fly, and in a few other insects. The fly has four pairs of chromosomes, and by some trick of Nature, each chromosome in the salivary gland can make about 1000 copies of itself during interphase. These copies associate in a side-by-side, parallel fashion to create a highly ordered structure that can be seen by means of the light microscope. Some very clear, detailed pictures of fly polytene chromosomes were shown in Fig. 1.4. Each individual chromosome retains its dispersed form, but there are so many copies of it in the polytene chromosome, all in register with one another, that the assemblage almost becomes visible to the naked eye, its overall size being about 1/30 mm.

Furthermore, these giant constructions show a great deal of substructure, in the form of well-defined regions of dark and light, or 'bands' and 'interbands'. The bands contain 95% of the total DNA plus protein, while the interbands contain 5%. Thus, DNA and protein must be packed together much more tightly in the bands than in the interbands, because both regions are of about the same size. It would be good to know how the bands and interbands come about. They provide some of the clearest evidence concerning the structural organisation of a chromosome between the level of the 300 Å fiber and that of the final, compact form which is seen only when a cell is on the point of dividing. Many precise details of the fly polytene chromosomes are now known, as a result of careful studies by

light and electron microscopy; but the structural basis for the band–interband organisation of DNA, which probably is determined somehow by the DNA base sequence itself, remains unclear.

In Fig. 6.3 we suggested that DNA might sometimes form 'loops' of size about 50 000 base-pairs, at somewhat regular intervals along the length of a chromosome. In such a model, each loop would contain an average of about 50 turns of 300 Å fiber, or 250 nucleosomes. (Incidentally, the thin curly line in Fig. 6.3 represents a string of nucleosomes, rather than the DNA itself.) It seems possible that the bands and interbands seen in polytene chromosomes are constructed from a series of such loops. Each loop would compact the DNA longitudinally, thereby providing for the dense packing of DNA and protein seen in the polytene 'bands'; while the intervals between loops would correspond to the 'interbands', where the packing of DNA and protein is much less dense. Of course, the individual loops themselves cannot be seen in the light microscope, because it takes 1000 nearly identical loops to make one band; but one can see these loops in other unusual sorts of chromosome, as we shall describe below.

Recall that the length of a DNA double-helical thread is reduced by a factor of about 40 when it wraps into a 300 Å fiber. Folding the 300 Å fiber into a series of loops will reduce its length by another factor of about 25, yielding a total compaction of about $40 \times 25 = 1000$, on going from free DNA to the dispersed, interphase form of a typical chromosome. So when a cell divides, it will only have to reduce the size of its chromosomes by a further factor of about 10, in order to reach the total compaction of 10 000-fold mentioned at the beginning of this chapter.

Now are these loops real, or are they just an attractive model, devized to explain phenomena which we cannot understand at present? There is much indirect evidence in favor of loops; for example the observation that DNA will fragment into pieces of somewhat regular size near 50 000 base-pairs, when chromosomes are degraded gently on a large scale. Fragments of size 300 000 base-pairs are also observed, as a turn or collection of six smaller loops. Giant loops of size 2 million base-pairs have even been suggested on the basis of indirect evidence, for the wrapping of DNA over very large distances in interphase nuclei.

Yet the most direct evidence in favor of such loops has long been known, and comes from the detailed study of so-called 'lampbrush' chromosomes, which are found in animals such as the frog or newt, in cells that are preparing to become egg cells. The loops are readily visible there for one simple reason: because genes along those loops are churning out huge amounts of RNA, in preparation for making a new frog egg or newt egg. Thus, those genes are covered almost

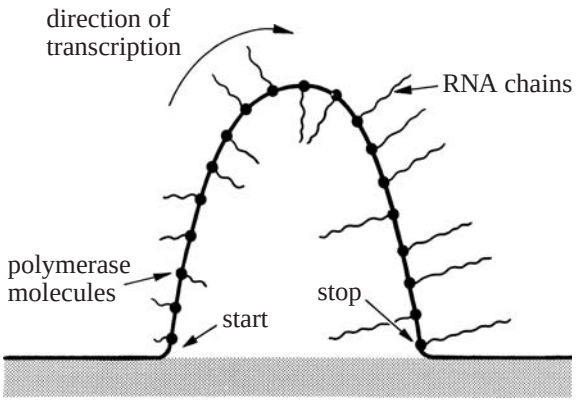

Figure 7.4 RNA polymerase molecules working their way around a loop of DNA in a lampbrush chromosome: schematic for both DNA and the base of the loop, or 'scaffold'. The RNA chains made by these polymerases grow longer as the polymerases travel further along the loop.

entirely by RNA polymerase molecules and their associated RNA chains, rather than by histone proteins. The DNA has lost almost all of its compaction due to wrapping about the histone proteins, and yet it remains relatively dense, owing to the great accumulation of protein and RNA along the length of any loop: so it is easy to see in the microscope.

As shown schematically in Fig. 7.4, the RNA polymerase molecules pack very densely along the length of the DNA in loops of a lampbrush chromosome, like cars in a queue at a traffic light. The RNA chains that emerge to either side are coated in protein (not shown in the diagram), and these chains grow longer as the polymerase molecules travel for greater distances around the loop. Each loop is anchored at its base in two places to certain unknown proteins (or other kinds of molecule) that provide a firm support or 'scaffold' for the flexible loop; and then there is an interval of some distance between loops, until another point of attachment to the scaffold is reached. These arrangements are rather similar to the 'band–interband' kinds of structure seen in fly polytene chromosomes, but in flies the loops become much more condensed, because they are covered in histone proteins.

Thus, in every case where we can actually see the fine structure of a chromosome by use of the light or electron microscope, we can see evidence for an intermediate level of structure between that of the 300 Å fiber and that of the folded metaphase chromosome. Furthermore, it is not unreasonable to suppose, as a working hypothesis, that this intermediate level of structure might consist simply of a series of large loops in the DNA, together with the intervals between loops.

But there are almost no certain data from biochemical studies today, to show which sequences in the DNA might attach themselves to the scaffold at the base of every loop, or to which proteins in such a scaffold the DNA might be attached.

A common procedure for investigating structures at this level, which has been followed by many workers, is to remove all the histones from the DNA by use of salt, detergent, or other reagents, and then to say that the proteins left represent a 'scaffold'. There are obvious dangers associated with this kind of approach, however, and the results remain controversial. For example, unless the DNA is attached to the scaffold more firmly than to the histones, the true attachments of DNA to any real scaffold might be lost during the treatment. Thus, in doing such an experiment, one might be left at the end with some sort of residual protein, rather than with any scaffold. A certain procedure devised cleverly by Uli Laemmli and colleagues uses only a relatively weak detergent to remove the histones, and does provide self-consistent evidence that the scaffold proteins which were left after treatment with such detergent, may have remained in their original places; but still the various procedures used are potentially destructive, as recent studies show. For example, Dean Jackson and Peter Cook have shown that the apparent size of 'loops' obtained by such methods is highly sensitive to the means of preparation of the sample. Furthermore, the loops as mapped by this kind of approach in fly polytene chromosomes do not show any correspondence to the band and interband structures that can be seen clearly by use of a light microscope. Finally, in one case it has been shown that a certain piece of DNA will attach itself to the scaffold only after, and not before, the addition of detergent or similar reagent. Still other studies show that topoisomerase II, the major proposed 'scaffold' protein which cannot be removed by detergent (probably because it binds covalently to the DNA while it alters the linking number Lk), does not play any sort of scaffolding or structural role when chromosomal loops are assembled in cell extracts.

Some interesting work on this difficult subject has been done in Siberia, by V.F. Semeshin, I.F. Zhimulev and colleagues. They have studied by electron microscopy the insertion of foreign bits of DNA into a fly polytene chromosome. For their foreign DNA, they used a special piece of DNA known as a 'P-element', that contains sequences which enable a fly enzyme to insert foreign DNA into fly DNA. They find that the P-element can either make a new band, or else split a previously existing band, depending upon its site of insertion in the chromosome. There are also other cases, not yet studied by electron microscopy, where the gene activity within a P-element can be 'insulated' from its position in a chromosome by

attaching certain DNA sequences to both sides. Normally the activity of any gene is highly sensitive to its position in a chromosome; yet these experiments suggest that certain sequences in the DNA can set up a 'boundary', possibly in the form of a loop-attachment site, between different genes on a chromosome.

All of the compact metaphase chromosomes, the fly polytene chromosomes, and the frog lampbrush loops are highly specialized structures in biology, adapted to particular functions. Most of the time any cell nucleus resides in interphase; so in order to understand how chromosomes really work, we need to dissect the structure and function of that dispersed state. In the nucleus at interphase, the various long chromosomal DNA molecules are not all tangled up together, but instead remain segregated from each other in different spatial domains. Within any one chromosome, regions of transcriptionally active and inactive chromatin are also separated in space. Certain inactive regions (called 'heterochromatin') seem to be associated with the tough nuclear membrane; while certain active regions of chromatin, involved in transcription, seem to lie often on the two edges of any individual chromosomal domain.

An important advance in understanding how this interphase nuclear 'architecture' might be established comes from a recent discovery, by Kohwi-Shigematsu and colleagues, of a cage-like network within the nucleus composed of a protein called SatB1. That protein binds preferentially to regions of DNA which easily unpair into single strands. SatB1 anchors many genes to specific chromosomal sites, and also tethers the bases of chromosomal loops. It can further recruit enzymes which alter the structure of chromatin; and thus it has all the required properties of a protein which might regulate the function of an authentic chromosomal domain.

By this point you may be feeling quite frustrated at the general air of uncertainty in our presentation! Ever since we stopped talking about nucleosomes, and went on to talk about the 300 Å fiber, HMG proteins, loops and scaffolds, there has hardly been anything definite to learn. But before we leave this topic, we shall mention one further aspect of the looping behavior of DNA in chromosomes, which seems to have a lot to do with how genes work; it illustrates only too well how fluid is our knowledge of these important matters. In Fig. 7.4 we implied that the loops of DNA in a chromosome might be rigidly fixed structures, because the DNA in each loop seems to be held at its two ends by a protein rod or scaffold. But only some of the scientists working on chromosomes today think in that way. Others think that the loops are flexible structures, which allow the DNA to slide or thread itself through the base of a chromosome, in the same way that a piece of magnetic tape goes through the reading-head of a tape recorder.

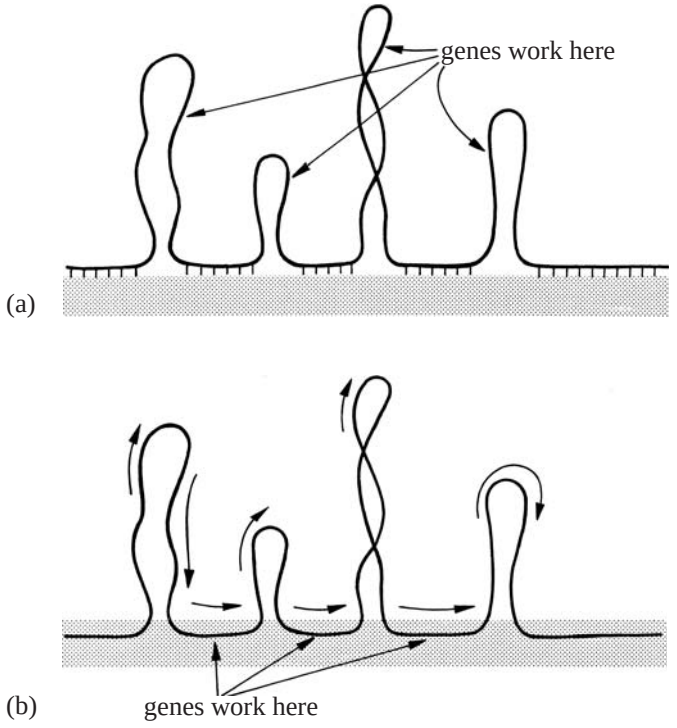

Figure 7.5 Two hypotheses for the location of gene activity in a typical chromosome from higher organisms, and here shown schematically. We do not know if the hypothetical loops of DNA are rigidly fixed structures as in (a), or if they are flexible enough to let the DNA slide through their sites of attachment to a 'scaffold' as in (b).

The two alternative models are sketched in Fig. 7.5. In Fig. 7.5(a), the loops are drawn as if they were fixed objects, and it is assumed that RNA polymerase travels along the genes which are contained in the outer parts of each loop. In other words, the polymerase and its associated proteins start making RNA near one end of the loop, and stop when (or before) they reach the other end. In Fig. 7.5(b), by contrast, the loops are able to slide through the base of the chromosome, where it is supposed that all of the polymerases and their associated proteins are stored in a kind of 'active compartment'. The DNA then threads itself through this active region so as to come into contact with the polymerase, and thus to make RNA.

At present, there is some evidence in support of each of these two theories. You must realize that biologists today are able to isolate many of the proteins that make genes work (the 'transcription factors'), but they do not yet know where these proteins are located – whether on the base or the tip of a chromosomal loop. On the one

hand, it is known that a polymerase molecule can track around the outer parts of a loop in special cases (see Fig. 7.4), when the genes are making very large quantities of RNA. On the other hand, some loops within a lampbrush chromosome are thought to be able to change size, as shown schematically in Fig. 7.5(b). Also, in relation to normal chromosomes that are not making large quantities of RNA, it has been reported that one can cut away 90% of the DNA and protein from the outer parts, to leave just the central part or 'scaffold'; and in the process one keeps almost all of the enzymatic activities needed for making RNA.

After this excursion into looping and gene activity, let us now return to better-understood subjects. On a much larger scale than we have considered so far, each chromosome has two kinds of specialized structure, which correspond to specific sequences in the DNA. These are known as the 'centromere' (or 'central part') and the 'telomeres' (or 'end parts'), as shown schematically in Fig. 7.6. The centromere lies somewhere within the main part of each chromosome, and it is the feature which becomes attached to the tubular protein structures, known as 'microtubules', that assemble themselves when the cell is about to divide. These microtubules pull the duplicated chromosomes apart, thus providing one copy of each chromosome for each new cell. In the photograph of Fig. 1.3, the two DNA centromeres lie within the narrow, central X-shaped part of each duplicated chromosome. The telomeres, on the other hand, lie at either end of a long, linear chromosome, and their role remains uncertain. Obviously they can 'seal' the ends of any chromosome to prevent its joining to other chromosomes; but they may well do more than that. Some scientists think that the telomeres adhere to the nuclear membrane, so as to anchor the chromosomes in three-dimensional space. Other people think that the telomeres are needed to assist in the copying of a linear chromosome upon cell division,

Figure 7.6 Special functional regions of chromosomal DNA. A centromere need not necessarily be located at the very center of the chromosomal DNA, but the telomeres always occupy the ends. Telomeres are short, repetitive DNA sequences such as TTAGGG, repeated hundreds of times in a row (in fish, frogs, and humans). Centromeres are long, semi-repetitive sequences of repeat-length at least 1000 to 10 000 base-pairs, that may provide for multiple attachment sites of DNA to microtubules. Many of the chromosomal centromeric regions have been characterized and isolated from simple organisms such as yeast, but no functional centromere has yet been isolated from any higher organism, with certainty.

because the usual copying enzyme (DNA polymerase) cannot copy DNA all the way to the end of a linear molecule, on both strands of the double helix (owing to its requirement for a small 'primer', see Chapter 10); hence a special enzyme, known as 'telomerase', copies the telomeric ends.

Let us now consider in more detail the structure of a compact, metaphase chromosome. Many studies by light and electron microscopy have shown that such duplicated chromosomes, which are ready to be separated into two parts on cell division (where one copy goes to each daughter-cell), have a sort of spiral structure as shown in Fig. 7.7(a). You can see there the same overall shape as in Fig. 1.3, but now more detail is visible. A thick rod or sausage-shape coils round and round to make spiral arms that serve to reduce the overall size of the chromosome to assist in cell division. Of what, precisely, might this thick rod be made? One obvious possibility would be the scaffold-and-loop structure which we have already described (Fig. 7.7(b)). The loops are small in this picture by comparison with those shown earlier, because the two diagrams in Fig. 7.7(b), which provide both side and end-views of the loops, are drawn on the scale of the much larger Fig. 7.7(a). Yet it seems possible that the bulk of the spiral rod may consist mainly of these loops, while the protein scaffold constitutes perhaps a relatively narrow core.

The formation of compact coils from a scaffold-and-loop structure would account nicely for the final compaction by a factor of 10, that is needed to achieve an overall compaction of 10 000 between the length of the free DNA and the length of a metaphase chromosome. It seems possible that the cell could make (hypothetically)

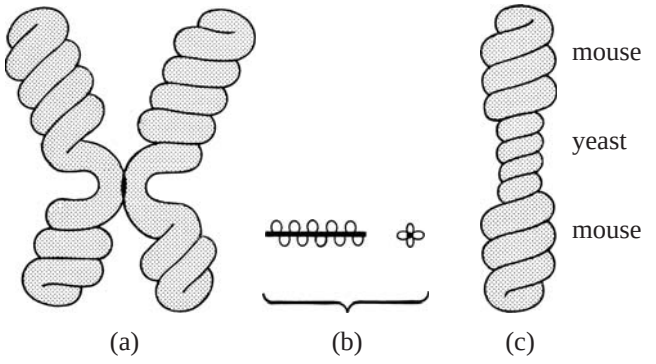

Figure 7.7 (a) Detailed fine-structure of the duplicated, metaphase chromosome as a wrapped-up spiral-rod or sausage-shape (cf. Fig. 1.3). (b) This same rod shown in detail as a hypothetical scaffold with loops, in two views. (c) A special mouse–yeast hybrid chromosome of two different diameters, revealing our ignorance of the factors that influence chromosome structure on a large scale.

some special protein that induces curvature in the scaffold-and-loop structure, as the cell gets ready to divide, thereby providing for the change from an interphase to a metaphase chromosome. Both left-handed and right-handed spirals – of the kind shown in Fig. 7.7(a) – have been seen by light and electron microscopy.

Scientists have recently found many different ways of transferring the DNA from one organism into the chromosomes of another. For example, the total DNA from a yeast chromosome of 9 million base-pairs can be inserted into the chromosome of a cell isolated from the mouse by a rare 'fusion' of the two kinds of cell in tissue culture. The resulting metaphase chromosome has a strange, dumb-bell shape (Fig. 7.7(c)): this picture is a simplified version, corresponding to one half of Fig. 7.7(a). The key feature is that the central part, which contains the yeast DNA, has only half the diameter of a normal mouse chromosome. One possible explanation is that the loops of yeast DNA are shorter than those of mouse DNA, thereby making the scaffold-and-loop structure smaller in diameter. Another possibility is that the protein scaffold in yeast curves into tighter spirals than does the scaffold in mouse. And perhaps both of these effects happen simultaneously. In any case this is a very deep result, because it reveals that there is a definite level of organisation in a metaphase chromosome, as specified by the sequence of DNA (or its pattern of methylation; see Chapter 11), which we do not currently understand.

Eventually, and probably in the present century, people will want to assemble full-length, authentic chromosomes in a test-tube, and use these in agriculture (and perhaps medicine) in order to do useful things that are not yet possible. Most present-day agricultural plants and farm animals are not wild species: instead, they are species that have been selectively bred for their food value by farmers over thousands of years. For example, the well-known 'Granny Smith' apple and the 'Parson Brown' orange were bred by people in the last century for improved quality of fruit. About twenty years ago, some scientists at the Calgene company in California made a tomato plant which produces tomatoes that will not rot, but will remain firm and red. This was done by adding to the tomato plant small amounts of foreign DNA which inhibit production of the 'rotting' enzyme. Since then, there have been many other examples of 'genetically modified' (GM) foods; for instance, cereals which incorporate a gene for insect resistance, or rice which is able to grow in salty water.

Much research is now in progress, aimed at developing methods for efficiently inserting large pieces of foreign DNA into cells, to make either 'transgenic' plants or animals for agriculture (for example, a transgenic pig that grows to maturity more rapidly than normal); or to deliver important genes to cells for 'gene therapy' in medicine, say

as a new treatment for cancer or as a means to correct genetic defects. These new methods often use viruses or fat-DNA complexes known as 'liposomes' for their delivery into cells, and they will be discussed in Chapter 10. Other research is now in progress, to make functional mini-chromosomes that will grow normally and be inherited in human cells. It is not known whether any of these methods will involve histones and DNA in their eventual mode of action; but it seems worthwhile to study the folding of DNA about histones at least as a model system, in order to understand more clearly this kind of process, and how it might be carried out in the cell or in a test tube.

So far, scientists have made only the smallest start at understanding the assembly of DNA into chromosomes. They would like to be able to put into a test-tube moderate quantities of pure DNA, pure protein, and perhaps other substances, in order to assemble chromosomes in the laboratory. But that sort of thing is still a long way off. At present, histones and DNA can only be combined to make authentic nucleosomes in the test-tube by adding 'extracts' taken from living cells. These extracts are presumed to contain many important factors for the assembly of nucleosomes, and perhaps for the assembly of loops and scaffolds; but it is not yet known exactly what these factors are, or how they work. Some of the extracts can even assemble a nuclear membrane around the DNA. There is a lot of interesting work to be done here.

The most important early result in this general area was obtained by Ron Laskey and colleagues in 1977. They found that one could incubate DNA with an extract from frog eggs, in order to make authentic fragments of a chromosome. Eventually they and Juergen Kleinschmidt independently found two proteins in the frog egg, called 'N1' and 'nucleoplasmin', that bind to the histone pairs H3, H4 and H2A, H2B, respectively, and carry them onto the DNA. Why should 'carriers' such as these be needed to place the histones onto the DNA in the form of nucleosomes? Why should histones not be able to bind to DNA spontaneously?

The water in our cells contains various dissolved salts, at low concentration. In the test-tube, histones only place themselves on DNA in the form of nucleosomes if the salt concentration is first made much higher than it is in our cells, and is then reduced slowly. At physiological, cell-like salt concentrations, the positively charged histones tend to aggregate in clumps, rather than form nucleosomes. But the carrier proteins N1 and nucleoplasmin contain many negatively charged amino acids, such as aspartate and glutamate; and these bind tightly to the histones so as to prevent them from aggregating. In fact, a simple polymer of aspartate or glutamate can also assemble histones onto DNA. Nature uses complicated proteins