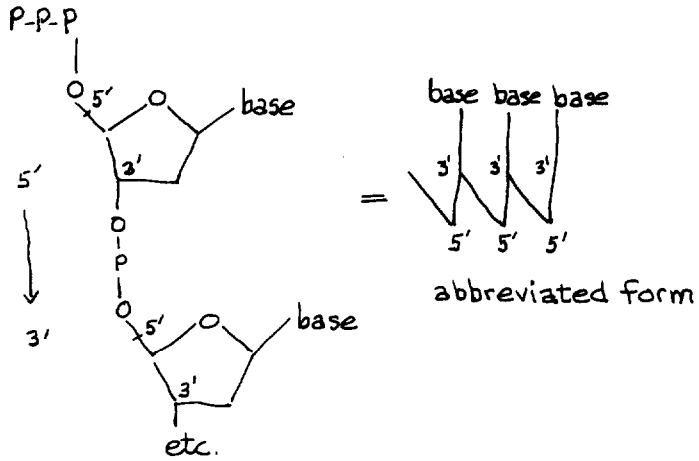
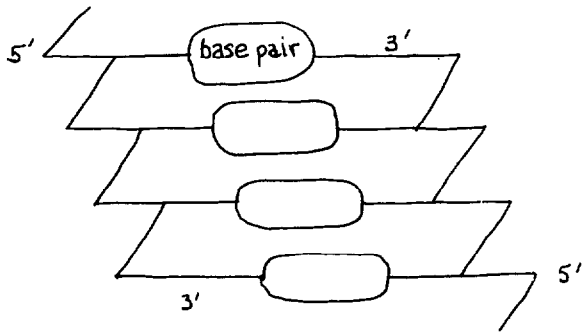
Lecture 11

Antiparallel synthesis

All synthesis of nucleic acids, whether DNA or RNA, consists of the same overall chemical process, in which nucleoside triphosphates serve as donors of nucleotides to build chains in the 5'-3' direction. The products of DNA or RNA synthesis consist of polynucleotide chains, which can be written in two ways:



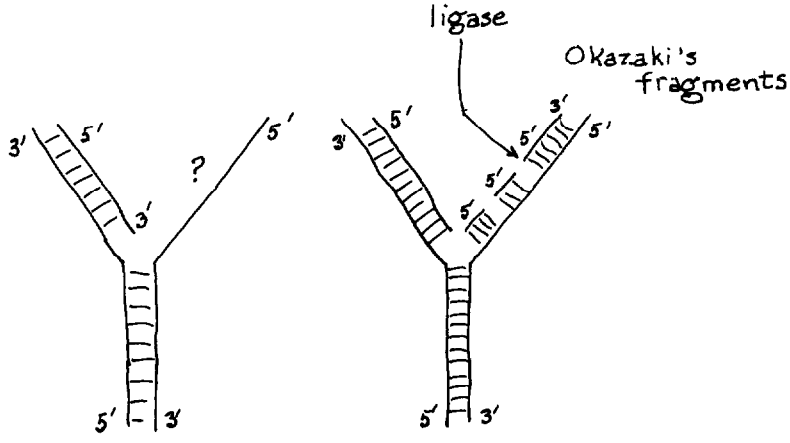
Each strand of DNA has a free 5' end (with or without one, or two, or three phosphates) at one end and a free 3' hydroxyl end. Because of the nonsymmetrical arrangement each strand has a polarity, either 5' → 3' or 3' → 5'. In a DNA molecule the two strands have opposite polarities:



The bases in one strand are upside-down with respect to the bases in the complementary strand. The only way two nucleotide strands can come together and form a double helix à la Watson and Crick is in this antiparallel way. There is no way to build them together to form a double helix

with parallel strands. More generally, whenever there is pairing between two nucleic acid strands (DNA-DNA, DNA-RNA, or RNA-RNA), it is always antiparallel. Thus there are two rules of pairing: (1) the normal base pairing always occurs between G and C and between A and T (or between A and U in RNA); and (2) the two strands are antiparallel.

This creates a curious difficulty. Both strands of a double-helix DNA are replicated in the same direction. Yet enzymes make DNA strands only in the direction 5' → 3'. How then can they replicate both strands? The 3' → 5' strand is easy to replicate, but how can the enzyme replicate the 5' → 3' strand, which would require building in the 3' → 5' direction?



A biochemist named Okazaki made a helpful discovery. By labeling a new DNA for only a few seconds, he found that the label was in very short pieces of DNA. These then disappear and are incorporated into longer pieces of DNA. So he proposed that synthesis of the 3' → 5' chain occurs through a backward formation of pieces in the 5' → 3' direction, which are then joined together. This joining could be done by a well-known enzyme, DNA ligase, which can do just that. In other terms, Okazaki's model is that one DNA strand is being made backwards. The Okazaki pieces may be the real way DNA is made; but this is not yet certain.

The problem with all these experiments is that DNA synthesis is a very fast process (1,000 nucleotides added per second at 30°C). The whole DNA of a cell like *E. coli*, over a million nucleotide

pairs long, can be replicated within 30 or 40 minutes.

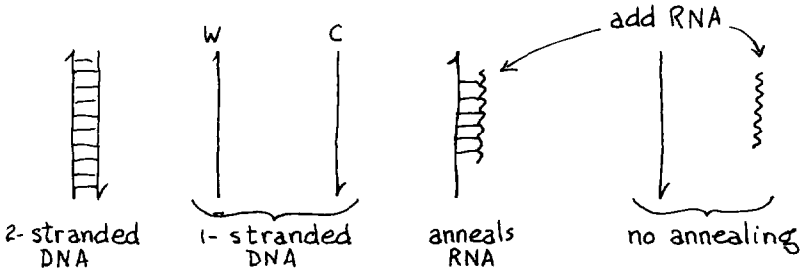
Annealing

What happens when the two strands in an intact double helical DNA molecule are separated but not replicated (for example, in making RNA)? The two strands can go back together to reform the original structure by a process called annealing. In the test tube, you can separate DNA strands by setting an alkaline pH or by heating. If you heat DNA in a salt solution to around 90°C, the strands separate because the thermal energy breaks the hydrogen bonds that held them together. If the solution is then cooled slowly down to room temperature, the correct double helical structure reforms. If instead the solution is cooled by quickly immersing it in ice, the double-stranded structure cannot reform because there has not been time for the trial-and-error collisions needed to regenerate the correct double strand structure. Small local regions of base-pairing may form within each strand (always in an anti-parallel way), but the strands will have the overall structure of a random coil.

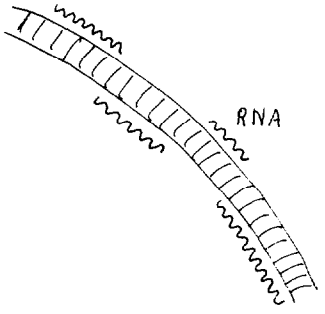
There are many ways to determine whether or not DNA has reannealed. Single-stranded DNA can be distinguished from double-stranded DNA by centrifugation, since the former, all balled up, sediments faster than the latter (more rigid, more elongated, subject to more friction). More convenient are methods based on the different behavior of single- and double-stranded nucleic acids in passing through filter membranes. Still another way is to use the electron microscope: a single-stranded DNA piece looks like a roundish mass, and a double strand looks like a string or thread. Also, there are enzymes that digest single-stranded but not double-stranded DNA and can be used to chew off single-stranded ends from a DNA duplex.

One-strand transcription

Sometimes, for example for some bacteriophages, the two strands of DNA, once separated, turn out to have different densities and can be physically separated: as someone put it, you can get Watson away from Crick. (The reason is that DNA density depends on the content of G, which is denser than the other bases.) This observation is important because it can answer the question, Which strand of DNA is the template for the corresponding RNA?



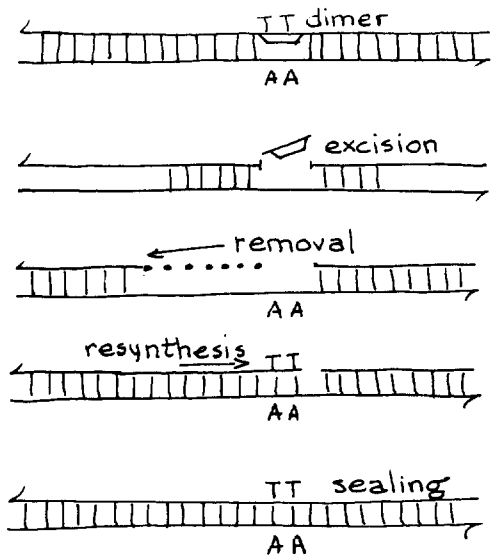
If you have some labeled RNA known to be made by a given piece of DNA, you can test the ability of the labeled RNA to anneal with each of the separated DNA strands. The RNA anneals only with the DNA strand to which it is complementary, that is, the strand whose transcript it is. The general conclusion is that, whenever RNA is produced it is transcribed off only one of the two DNA strands. For example, in the E. coli chromosome, RNA may be transcribed from one strand in some places, the other strand in other places, but never off both strands at the same site.



Is this situation reasonable? Could evolution have produced double, or W-C, genes, in which one DNA strand is coded for one product and the complementary strand, read in the opposite direction, coded for a different polypeptide product? There is no a priori reason against it except for the problem of mutations. If both strands carried genetic information, every mutation would result in a change in two genes. This is an enormously unfavorable situation, sufficient in itself to discourage the evolution of W-C genes. Since most mutations are unfavorable, evolution must have preferred to waste all of the information in the unused segments of DNA strands rather than risk the chance of being messed up genetically.

DNA repair

I mentioned earlier the ligase enzyme, which is present in every cell. It might be there just to please Okazaki. But it has at least one other important function. The ligase is part of a group of enzymes that repairs DNA whenever it is damaged, for example, by ultraviolet light or by x rays or by mistakes in replication. Every normal living cell has such a system for repairing DNA. For example, ultraviolet light can cause two adjacent thymine bases in a DNA strand to join in a way that would prevent DNA from replicating. This is called a thymine dimer. The repair system starts working in a series of steps:



1. an enzyme breaks the damaged strand of DNA on either side of the joined bases;
2. another enzyme chews up a small piece of the DNA on one side of the damaged section;
3. a third enzyme, polymerase I, rebuilds the missing piece again using the opposite strand as a template;
4. the ligase seals the break.

There is a human genetic disease called xeroderma; the affected people lack one of these repair enzymes. They can live well, but if they get exposed to too much sunlight their skin cells are easily damaged and tend to produce cancers. The defect is in the repair system, which works badly. Its mistakes can lead to cancer by mutating one or more genes.

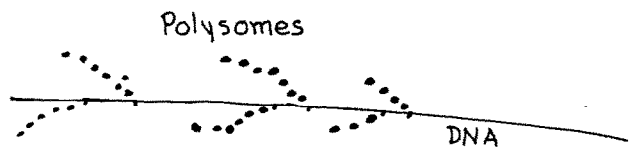
RNA synthesis

Let us now consider the synthesis of RNA. RNA is made from riboside triphosphates, usually by base pairing with one strand of DNA. The mechanism is conservative since the RNA comes off and the DNA strands return together. The RNA made in any cell falls into three classes:

1. Ribosomal RNA. A ribosome, part of the machine to make protein, consists of two subunits. Each subunit contains one molecule of RNA, always the same in a given organism. Bacterial ribosomes differ somewhat in size from those of animal cells.
2. Transfer RNA or amino acid adapters. These molecules are 70 to 85 nucleotides long and are specific for each individual amino acid. They

serve to insert the amino acids into proteins.
3. Messenger RNA. These are of variable size because they represent the transcript of individual genes or groups of genes, which will then direct the synthesis of proteins.
Thus all species of RNA participate in one way or another in protein synthesis.

The overall process of RNA transcription from DNA and its translation into protein in bacteria has been visualized by Oscar Miller in some superb electron microscopic pictures which look something like this:



This is the appearance of nascent strands of E. coli RNA still being made on template DNA. The long central fiber is the DNA and the fibers coming off it are the partially synthesized RNA molecules. The small particles visible on all of the RNA fibers are ribosomes. As soon as the messenger is even partially available, the ribosomes attach themselves to it and begin making protein. One molecule of mRNA may have up to 10 or 20 ribosomes attached at a given time, each making a polypeptide chain. Sometimes, however, there are no ribosomes attached to the nascent RNA strands. These RNA molecules do not code for protein, that is, they are either tRNA or rRNA.

The picture of RNA synthesis in animal cells is different: ribosomes are not visible on nascent RNA. This is because in eucaryotic cells messenger RNA picks up ribosomes and makes proteins only after it passes from the nucleus to the cytoplasm.

RNA polymerase

In every cell there is at least one enzyme, called RNA polymerase, that catalyzes the synthesis of RNA off of the DNA in the usual 5' → 3' direction. Molecules of RNA polymerases may be

seen in Miller's electron micrographs where RNA is still attached to DNA. This enzyme must be able to recognize the starting signals that indicate the beginning points of sections of the DNA that are transcribed into RNA molecules. RNA polymerase is the key enzyme for the expression of gene function. Genes are stretches of DNA; the polymerase recognizes where to begin and where to end on the DNA to make the RNA transcript of a gene or a group of genes.

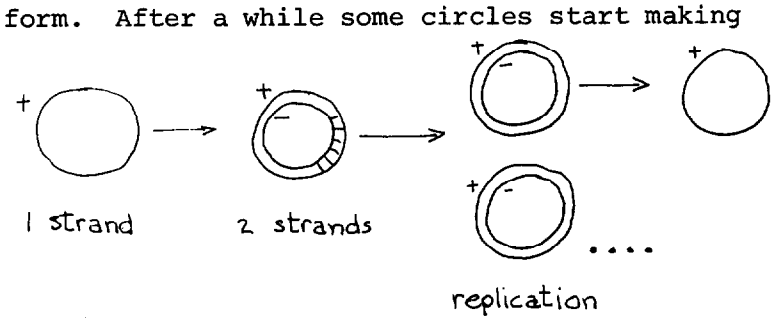
The above picture of nucleic acid synthesis is fairly straightforward. By the intervention of one or a few enzymes, DNA is replicated to generate more DNA. By the action of RNA polymerase DNA segments are transcribed into RNA molecules.

Viruses

There are some organisms, however, that include special cases with respect to the nature and replication of their genetic materials. These organisms are the viruses. Viruses are not cells; they must use living cells of other organisms to reproduce. Viruses, when in the free state, are in the form of particles or virions that consist of a piece of genetic material--a nucleic acid molecule--plus one or more protein coats. All viruses have some kind of mechanism for inserting their nucleic acid into specific host cells, where it is replicated and more virus is made. Viruses that grow in bacteria are called bacteriophages or phages.

Some viruses contain double-stranded DNA as genetic molecules; in principle, therefore, they present no difficulty. Their DNA can be replicated as usual using either the cell's machinery or sometimes by using a special viral machinery.

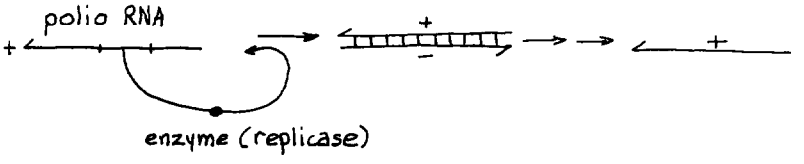
Some viruses, however, contain odd types of nucleic acid molecules. One class has a circular single-stranded DNA. When this enters a cell, an enzyme present in the cell, probably DNA polymerase I, makes a complementary strand so that the single-strand circle becomes a double-strand circle, which then proceeds to reproduce in the 2-strand form. After a while some circles start making



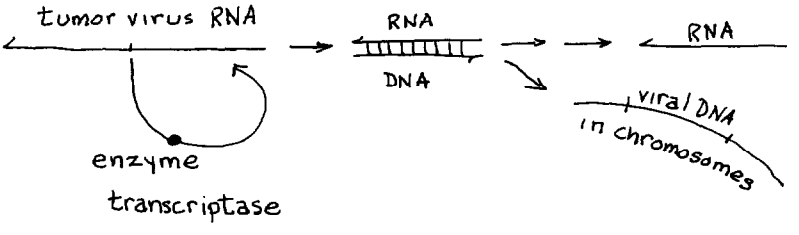
single-stranded DNA circles, which enter into complete viruses. You may have guessed that this mechanism is arranged in such a way that it always selects the same strand--Watson or Crick--to put in the complete virus particle. But how this happens we do not know.

RNA viruses

Other viruses, for example, poliovirus and some phages, are even odder: their genetic material is RNA! This raises a major difficulty. Cells, as far as we know, contain no machinery to make RNA from RNA or to make DNA from RNA. Some RNA viruses, such as polio, solve this problem in a direct way: the entering RNA, acting as mRNA, makes an enzyme that can copy the viral RNA, first making a complementary strand and then more viral RNA strands.



The most complicated and most exciting case is that of RNA tumor viruses. They are the cause of many cancers of animals and probably also of man. Their virions contain, besides single-stranded RNA, an enzyme called reverse transcriptase, which can make DNA from RNA! When the RNA of a tumor virus enters a cell, this enzyme comes in with it and catalyzes the synthesis of a complementary DNA strand, thus creating a DNA-RNA hybrid molecule.



The discovery of reverse transcriptase (in 1970) was a shock because it had been generally assumed (without any strong theoretical reasons) that in macromolecular syntheses information would flow only from DNA to RNA and from RNA to protein.

[One should not take beliefs too seriously. In August 1901 the famous philosopher Hegel wrote in his doctoral dissertation that the planets then known were of necessity the sum total of the solar planetary system. Unfortunately, the preceding January the astronomer Piazzi had discovered Ceres, and Neptune and Pluto were soon to follow. As Francis Crick has pointed out, he and Watson never postulated that information had to flow only from DNA to RNA rather than from RNA to DNA, but only that protein should not be able to serve as template to make nucleic acid. Can you see why?]

Reverse transcriptase is also important in one practical respect. It provides tumor viruses with a mechanism for converting their genetic material into DNA. This DNA can then remain in the cell, joining the DNA of the chromosomes. The permanent presence of viral DNA in cellular chromosomes is believed to be responsible for the transformation of normal cells to cancer cells by these viruses and possibly for many so-called spontaneous cancers.

Further reading

Watson's Chapter 11, "The Transcription of RNA upon DNA Templates," and Stent's Chapter 16, "DNA Transcription," provide relevant information beyond what is presented in this lecture.