In some viruses the flow of information is in reverse direction, that is, from RNA to DNA. *Can you suggest a simple name to the process?*

6.1.2 Packaging of DNA Helix

Taken the distance between two consecutive base pairs as 0.34 nm (0.34×10^{-9} m), if the length of DNA double helix in a typical mammalian cell is calculated (simply by multiplying the total number of bp with distance between two consecutive bp, that is, 6.6×10^9 bp × 0.34×10^{-9} m/bp), it comes out to be approximately 2.2 metres. A length that is far greater than the dimension of a typical nucleus (approximately 10^{-6} m). How is such a long polymer packaged in a cell?

If the length of E. coli DNA is 1.36 mm, can you calculate the number of base pairs in E.coli?

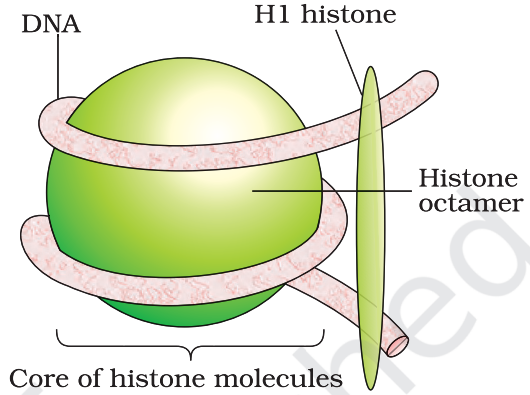

Figure 6.4a Nucleosome

In prokaryotes, such as, *E. coli*, though they do not have a defined nucleus, the DNA is not scattered throughout the cell. DNA (being negatively charged) is held with some proteins (that have positive charges) in a region termed as 'nucleoid'. The DNA in nucleoid is organised in large loops held by proteins.

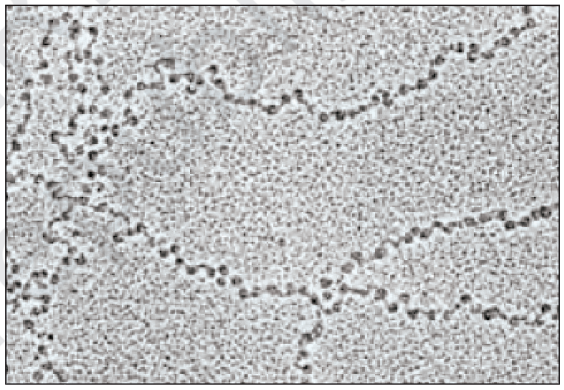

Figure 6.4b EM picture - 'Beads-on-String'

In eukaryotes, this organisation is much more complex. There is a set of positively charged, basic proteins called **histones**. A protein acquires charge depending upon the abundance of amino acids residues with charged side chains. Histones are rich in the basic amino acid residues lysine and arginine. Both the amino acid residues carry positive charges in their side chains. Histones are organised to form a unit of eight molecules called **histone octamer**. The negatively charged DNA is wrapped around the positively charged histone octamer to form a structure called **nucleosome** (Figure 6.4 a). A typical nucleosome contains 200 bp of DNA helix. Nucleosomes constitute the repeating unit of a structure in nucleus called **chromatin,** thread-like stained (coloured) bodies seen in nucleus. The nucleosomes in chromatin are seen as 'beads-on-string' structure when viewed under electron microscope (EM) (Figure 6.4 b).

Theoretically, how many such beads (nucleosomes) do you imagine are present in a mammalian cell?

The beads-on-string structure in chromatin is packaged to form chromatin fibers that are further coiled and condensed at metaphase stage of cell division to form chromosomes. The packaging of chromatin at higher level requires additional set of proteins that collectively are referred to as

Non-histone Chromosomal (NHC) proteins. In a typical nucleus, some region of chromatin are loosely packed (and stains light) and are referred to as **euchromatin**. The chromatin that is more densely packed and stains dark are called as **Heterochromatin**. Euchromatin is said to be transcriptionally active chromatin, whereas heterochromatin is inactive.

6.2 The Search for Genetic Material

Even though the discovery of nuclein by Meischer and the proposition for principles of inheritance by Mendel were almost at the same time, but that the DNA acts as a genetic material took long to be discovered and proven. By 1926, the quest to determine the mechanism for genetic inheritance had reached the molecular level. Previous discoveries by Gregor Mendel, Walter Sutton, Thomas Hunt Morgan and numerous other scientists had narrowed the search to the chromosomes located in the nucleus of most cells. But the question of what molecule was actually the genetic material, had not been answered.

Transforming Principle

In 1928, Frederick Griffith, in a series of experiments with *Streptococcus pneumoniae* (bacterium responsible for pneumonia), witnessed a miraculous transformation in the bacteria. During the course of his experiment, a living organism (bacteria) had changed in physical form.

When *Streptococcus pneumoniae* (pneumococcus) bacteria are grown on a culture plate, some produce smooth shiny colonies (S) while others produce rough colonies (R). This is because the S strain bacteria have a mucous (polysaccharide) coat, while R strain does not. Mice infected with the S strain (virulent) die from pneumonia infection but mice infected with the R strain do not develop pneumonia.

S strain ⟶ Inject into mice ⟶ Mice die

R strain ⟶ Inject into mice ⟶ Mice live

Griffith was able to kill bacteria by heating them. He observed that heat-killed S strain bacteria injected into mice did not kill them. When he

S strain (heat-killed) ⟶ Inject into mice ⟶ Mice live

S strain (heat-killed) + R strain (live) ⟶ Inject into mice ⟶ Mice die

injected a mixture of heat-killed S and live R bacteria, the mice died. Moreover, he recovered living S bacteria from the dead mice.

He concluded that the R strain bacteria had somehow been **transformed** by the heat-killed S strain bacteria. Some 'transforming principle', transferred from the heat-killed S strain, had enabled the R strain to synthesise a smooth polysaccharide coat and become virulent. This must be due to the transfer of the genetic material. However, the biochemical nature of genetic material was not defined from his experiments.

Biochemical Characterisation of Transforming Principle

Prior to the work of Oswald Avery, Colin MacLeod and Maclyn McCarty (1933-44), the genetic material was thought to be a protein. They worked to determine the biochemical nature of 'transforming principle' in Griffith's experiment.

They purified biochemicals (proteins, DNA, RNA, etc.) from the heat-killed S cells to see which ones could transform live R cells into S cells. They discovered that DNA alone from S bacteria caused R bacteria to become transformed.

They also discovered that protein-digesting enzymes (proteases) and RNA-digesting enzymes (RNases) did not affect transformation, so the transforming substance was not a protein or RNA. Digestion with DNase did inhibit transformation, suggesting that the DNA caused the transformation. They concluded that DNA is the hereditary material, but not all biologists were convinced.

Can you think of any difference between DNAs and DNase?

6.2.1 The Genetic Material is DNA

The unequivocal proof that DNA is the genetic material came from the experiments of Alfred Hershey and Martha Chase (1952). They worked with viruses that infect bacteria called bacteriophages.

The bacteriophage attaches to the bacteria and its genetic material then enters the bacterial cell. The bacterial cell treats the viral genetic material as if it was its own and subsequently manufactures more virus particles. Hershey and Chase worked to discover whether it was protein or DNA from the viruses that entered the bacteria.

They grew some viruses on a medium that contained radioactive phosphorus and some others on medium that contained radioactive sulfur. Viruses grown in the presence of radioactive phosphorus contained radioactive DNA but not radioactive protein because DNA contains phosphorus but protein does not. Similarly, viruses grown on radioactive sulfur contained radioactive protein but not radioactive DNA because DNA does not contain sulfur.

Radioactive phages were allowed to attach to *E. coli* bacteria. Then, as the infection proceeded, the viral coats were removed from the bacteria by agitating them in a blender. The virus particles were separated from the bacteria by spinning them in a centrifuge.

Bacteria which was infected with viruses that had radioactive DNA were radioactive, indicating that DNA was the material that passed from the virus to the bacteria. Bacteria that were infected with viruses that had radioactive proteins were not radioactive. This indicates that proteins did not enter the bacteria from the viruses. DNA is therefore the genetic material that is passed from virus to bacteria (Figure 6.5).

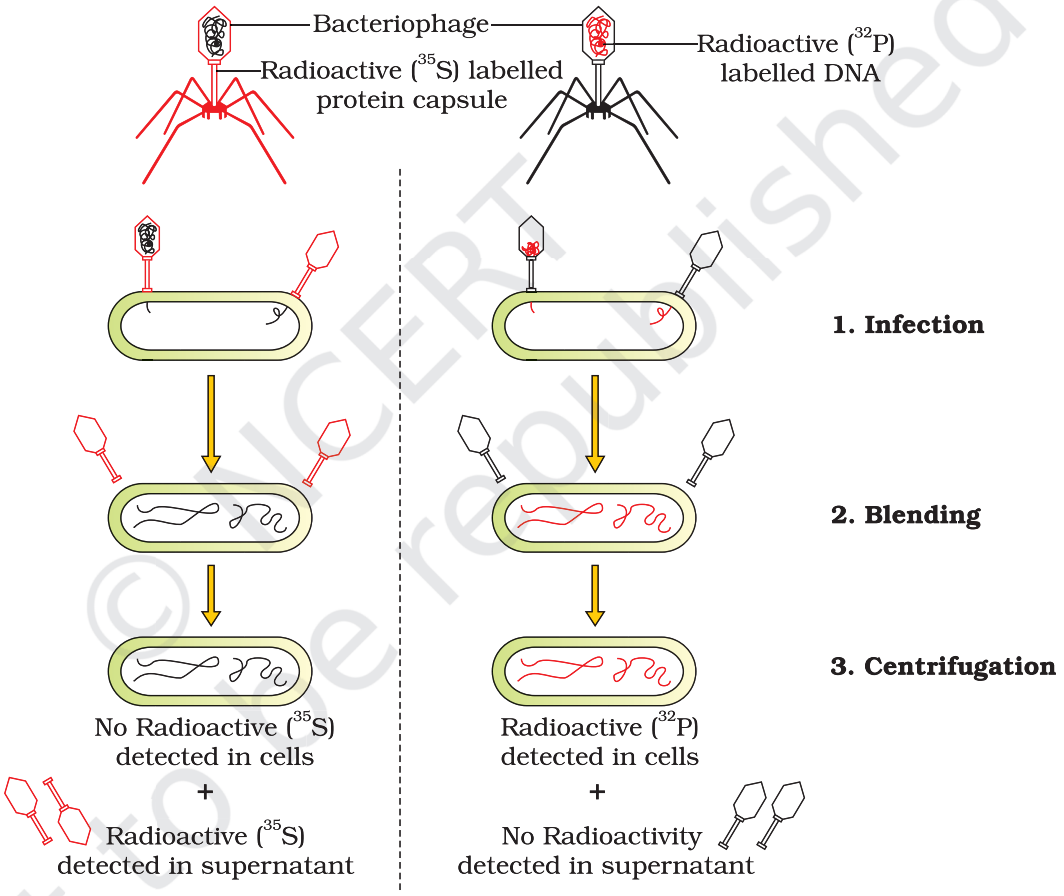

Figure 6.5 The Hershey-Chase experiment

6.2.2 Properties of Genetic Material (DNA versus RNA)

From the foregoing discussion, it is clear that the debate between proteins versus DNA as the genetic material was unequivocally resolved from Hershey-Chase experiment. It became an established fact that it is DNA that acts as genetic material. However, it subsequently became clear that

in some viruses, RNA is the genetic material (for example, Tobacco Mosaic viruses, QB bacteriophage, etc.). Answer to some of the questions such as, why DNA is the predominant genetic material, whereas RNA performs dynamic functions of messenger and adapter has to be found from the differences between chemical structures of the two nucleic acid molecules.

Can you recall the two chemical differences between DNA and RNA?

A molecule that can act as a genetic material must fulfill the following criteria:

(i) It should be able to generate its replica (Replication).

(ii) It should be stable chemically and structurally.

(iii) It should provide the scope for slow changes (mutation) that are required for evolution.

(iv) It should be able to express itself in the form of 'Mendelian Characters'.

If one examines each requirement one by one, because of rule of base pairing and complementarity, both the nucleic acids (DNA and RNA) have the ability to direct their duplications. The other molecules in the living system, such as proteins fail to fulfill first criteria itself.

The genetic material should be stable enough not to change with different stages of life cycle, age or with change in physiology of the organism. Stability as one of the properties of genetic material was very evident in Griffith's 'transforming principle' itself that heat, which killed the bacteria, at least did not destroy some of the properties of genetic material. This now can easily be explained in light of the DNA that the two strands being complementary if separated by heating come together, when appropriate conditions are provided. Further, 2′-OH group present at every nucleotide in RNA is a reactive group and makes RNA labile and easily degradable. RNA is also now known to be catalytic, hence reactive. Therefore, DNA chemically is less reactive and structurally more stable when compared to RNA. Therefore, among the two nucleic acids, the DNA is a better genetic material.

In fact, the presence of thymine at the place of uracil also confers additional stability to DNA. (Detailed discussion about this requires understanding of the process of repair in DNA, and you will study these processes in higher classes.)

Both DNA and RNA are able to mutate. In fact, RNA being unstable, mutate at a faster rate. Consequently, viruses having RNA genome and having shorter life span mutate and evolve faster.

RNA can directly code for the synthesis of proteins, hence can easily express the characters. DNA, however, is dependent on RNA for synthesis of proteins. The protein synthesising machinery has evolved around RNA. The above discussion indicate that both RNA and DNA can function as