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First Genetic Material.. Reactive Catalyst

GROUPS/ DSC SPECIAL

BIOLOGY

RNA

- RNA was the first genetic material. There is now enough evidence to suggest that essential life processes (such as metabolism, translation, splicing, etc.), evolved around RNA. RNA used to act as a genetic material as well as a catalyst (there are some important biochemical reactions in living systems that are catalysed by RNA catalysts and not by protein enzymes). But, RNA being a catalyst was reactive and hence unstable. Therefore, DNA has evolved from RNA with chemical modifications that make it more stable.
- DNA being double stranded and having complementary strand further resists changes by evolving a process of repair.

REPLICATION

- While proposing the double helical structure for DNA, Watson and Crick had immediately proposed a scheme for replication of DNA. To quote their original statement that is as follows "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material" (Watson and Crick, 1953).
- The scheme suggested that the two strands would separate and act as a template for the synthesis of new complementary strands. After the completion of replication, each DNA molecule would have one parental and one newly synthesised strand. This scheme was termed as semiconservative DNA replication.

The Experimental Proof

- It is now proven that DNA replicates emiconserva tively. It was shown first in Esche richia coli and subsequently in higher organisms, such as plants and human cells. Matthew Meselson and Franklin Stahl performed the following experiment in 1958:

(i) They grew E. coli in a medium containing 15NH4Cl (15N is the heavy isotope of nitrogen) as the only nitrogen source for many generations. The result was that 15N was incorporated into newly synthesised DNA (as well as other nitrogen containing compounds).

- This heavy DNA molecule could be distinguished from the normal DNA by centrifugation in a cesium chloride (CsCl) density gradient (Please note that 15N is not a radioactive isotope, and it can be separated from 14N only based on densities).

(ii) Then they transferred the cells into a medium with normal

- $14NH_4Cl$ and took samples at various definite time intervals as the cells multiplied, and extracted the DNA that remained as double-stranded helices.
- The various samples were separated independently on CsCl gradients to measure the densities of DNA

(iii) Thus, the DNA that was extracted from the culture one generation after the transfer from 15N to 14N medium [that is after 20 minutes; E. coli divides in 20 minutes] had a hybrid or intermediate density.

- DNA extracted from the culture after another generation [that is after 40 minutes, II generation] was composed of equal amounts of this hybrid DNA and of 'light' DNA.
- If E. coli was allowed to grow for 80 minutes then what would be the proportions of light and hybrid densities DNA molecule?
- Very similar experiments involving use of radioactive thymidine to detect distri bution of newly synthesised DNA in the chromosomes was performed on Vicia faba (faba beans) by Taylor and colleagues in 1958.
- The experiments proved that the DNA in chromosomes also replicate semiconservati vely.

The Machinery and the Enzymes

- In living cells, such as E. coli, the process of replication requires a set of catalysts (enzymes). The main enzyme is referred to as DNA-depen dent DNA polymerase, since it uses a DNA template to catalyse the polymerisation of deoxynu cleotides. These enzymes are highly efficient enzymes as they have to catalyse polymerisation of a large number of nucleotides in a very short time. E. coli that has only 4.6 ×106 bp (compare it with human whose diploid content is 6.6 × 109 bp), completes the process of replication within 18 minutes; that means the average rate of polyme risation has to be approxi mately 2000 bp per second. Not only do these polymerases have to be fast, but they also have to catalyse the reaction with high degree of accuracy.
- Any mistake during replication would result into mutations. Furthermore, energetically replication is a very expensive process. Deoxyri bonucleoside tripho sphates serve dual purposes. In addition to acting as substrates, they provide energy for polymerisation reaction (the two terminal phosphates in a deoxynu cleoside ripho sphates are high-energy phosphates, same as in case of ATP).
- In addition to DNA-depen dent DNA polymerases, many additional enzymes are required to complete the process of replication with high degree of accuracy. For long DNA molecules, since the two strands of DNA cannot be separated in its entire length (due to very high energy requirement), the replication occur within a small opening of the DNA helix, referred to as repli cation fork. The DNA-depen dent DNA polymerases catal yse polymerisation only in one direction, that is 5'→3' . This creates some additional complications at the replicating fork.
- Consequently, on one strand (the tem plate with polarity 3'→5'), the repli cation is cont inuous, while on the other (the templ ate with polarity 5'→3'), it is discontinuous.
- The disconti nuously synthesised frag ments are later joined by the enzyme DNA ligase (Figure 6.8). The DNA polymerases on their own cannot initiate the process of replication. Also the replication does not init iate randomly at any place in DNA. There is a definite region in E. coli DNA where the replication originates. Such regions are termed as origin of replication. It is because of the requirement of the origin of replication that a piece of DNA if needed to be propagated during recombinant DNA procedures, requires a vector. The vectors provide the origin of replication.
- Further, not every detail of replication is understood well. In eukaryotes, the replication of DNA takes place at S-phase of the cell-cycle. The replication of DNA and cell division cycle should be highly coordinated. A failure in cell division after
- DNA replication results into polyploidy(a chromo somal anomaly). You will learn the detailed nature of origin and the processes occurring at this site, in higher classes.