Section B and C

Volume-05

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3. FUNDAMENTAL PROCESSES
A. DNA REPLICATION, REPAIR AND RECOMBINATION
1. DNA REPLICATION

DNA REPLICATION IN PROKARYOTES

Early Models for DNA Replication

In Watson and Crick's double-helix model for DNA, they reasoned that replication of the DNA would be straightforward if their model was correct. That is, by unwinding the DNA molecule and separating the two strands, each strand could be a template for the synthesis of a new, complementary strand of DNA. As the DNA double helix is progressively unwound from one end, the base sequence of the new strand would be determined by the base sequence of the template strand, following complementary base-pairing rules. When replication is completed, there would be two progeny DNA double helices, each consisting of one parental DNA strand and one new DNA strand. This model for DNA replication is known as the semi-conservative model since each progeny molecule retains one of the parental strands.

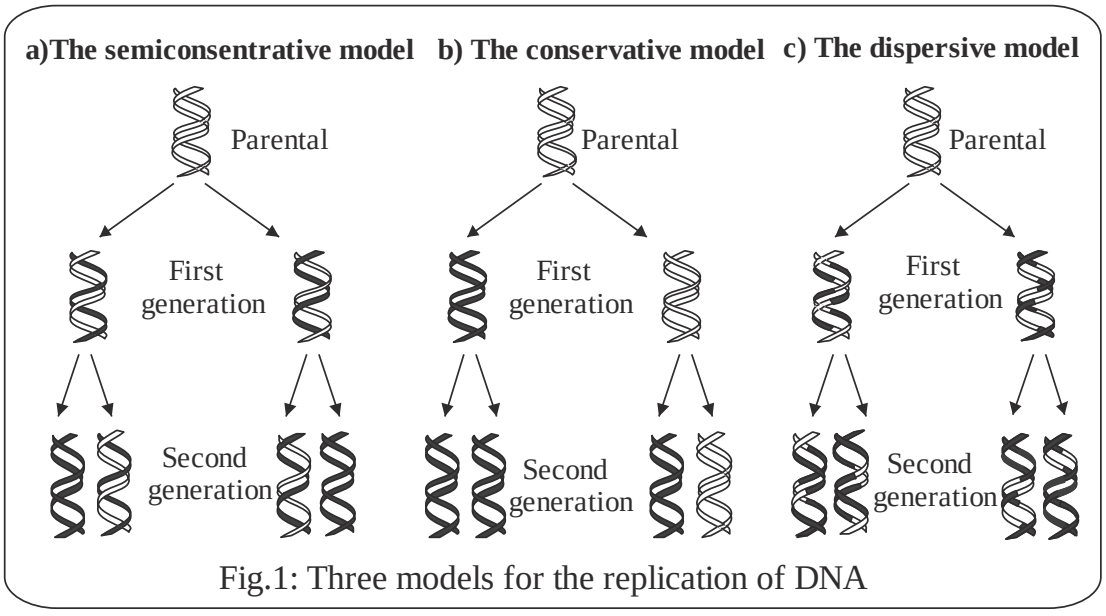

Fig.1: Three models for the replication of DNA

At the time, two other models for DNA replication were proposed, the conservative model and the dispersive model. In the conservative model, the two parental strands of DNA remain together or re-anneal after replication and as a whole serve as a template for the synthesis of new progeny DNA double helices. Thus, one of the two progeny DNA molecules is actually the parental double-stranded DNA molecule, and the other consists of totally new material. In the dispersive model, the parental double helix is cleaved into double-stranded DNA segments which act as templates for the synthesis of new double-stranded DNA segments. Somehow, the segments reassemble into complete DNA double helices, with parental and progeny DNA segments interspersed. Thus, while the two progeny DNAs are identical with respect to base pair sequence, the parental DNA has actually become dispersed throughout both progeny molecules.

The Meselson-Stahl Experiment

Five years after Watson and Crick developed their model, Matthew Meselson and Frank Stahl obtained experimental evidence that the semiconservative replication model was correct. To examine the process of DNA replication, Meselson and Stahl used the bacterium *Escherichia coli* because it can be grown easily and quickly in a minimal medium. In Meselson and Stahl's experiment, *E. coli* was grown for several generations in a minimal medium in which the only nitrogen source was $^{15}NH_4Cl$ (ammonium chloride).

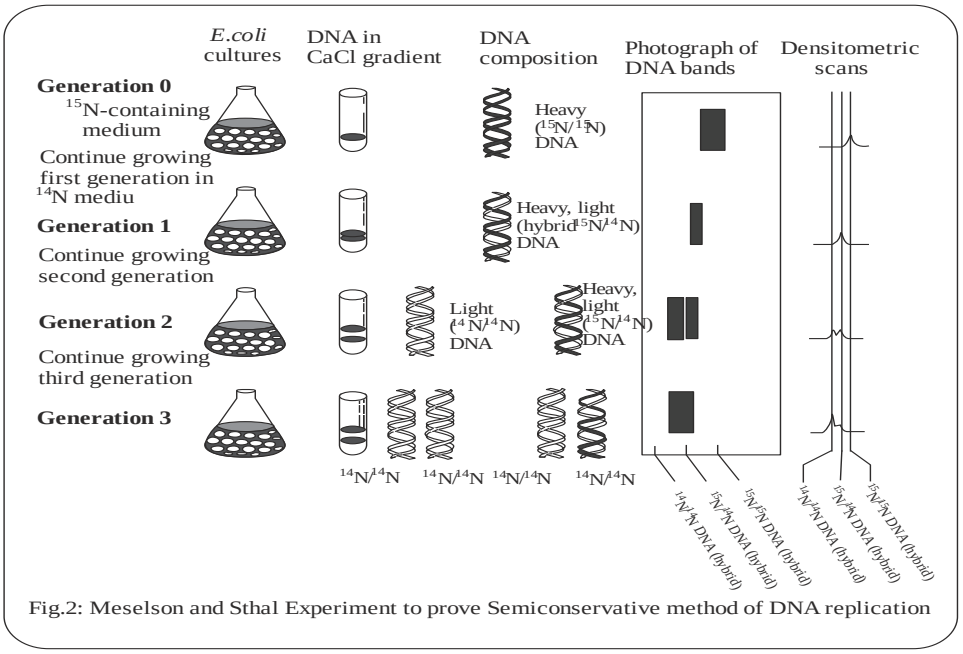

Fig.2: Meselson and Sthal Experiment to prove Semiconservative method of DNA replication

In this compound the normal isotope of nitrogen, ^{15}N, is replaced with ^{15}N, the heavy isotope. (Note: Density is weight/volume so ^{15}N, with one extra neutron in its nucleus, is more denser than ^{14}N.) As a result, all the bacteria's cellular nitrogen-containing compounds, including every purine and pyrimidine base in its DNA, contained ^{15}N instead of ^{14}N. ^{15}N DNA can be separated from ^{14}N DNA by using equilibrium density gradient centrifugation. Briefly, in this technique a solution of Cesium Chloride (CsCl) is centrifuged at high speed, causing the cesium chloride to form a density gradient. If DNA is present in the solution, it will band to a position where its buoyant density is the same as that of the surrounding cesium chloride.

Equilibrium density gradient centrifugation

In experiments involving equilibrium density gradient centrifugation, a concentrated solution of cesium chloride (CsCl) is centrifuged at high speed. The opposing forces of sedimentation and diffusion produce a stable, linear concentration gradient of the CsCl. The actual densities of CsCl at the extremes of the gradient are related to the CsCl concentration that is centrifuged.

For example, to examine DNA of density 1.70 g/cm^3 (a typical density for DNA), one makes a gradient that spans that density, for example from 1.60 to 1.80 g/cm^3. If the DNA is

mixed with the CsCl when the density gradient is generated in the centrifuge, the DNA will come to equilibrium at the point in the gradient where its buoyant density equals the density of the surrounding CsCl. The DNA is said to have banded in the gradient. If DNAs are present that have different densities, as is the case with ^{15}N-DNA and ^{14}N-DNA, then they will band (i.e., come to equilibrium) in different positions. The DNA is detected as UV-absorbing material. The method can also be used experimentally to purify DNAs on the basis of their different densities. It has been used, for example, to fractionate nuclear and mitochondrial DNA in extracts of eukaryotic cells and to separate bacterial plasmid DNA from the host bacterium's DNA.

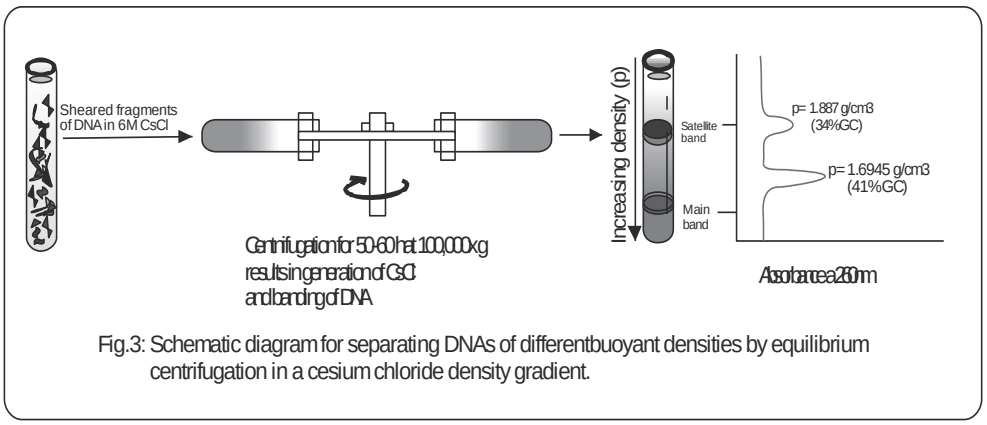

Fig.3: Schematic diagram for separating DNAs of different buoyant densities by equilibrium centrifugation in a cesium chloride density gradient.

As the next step in Meselson and Stahls experiment, the ^{15}N-labeled bacteria were transferred to a medium containing nitrogen in the normal ^{14}N form. The bacteria were allowed to replicate in the new conditions for several generations. Throughout the period of growth in the ^{15}N medium, samples of *E. coli* were taken, lysed to release the cellular contents, and the DNA was analyzed in cesium chloride density gradients. After one generation in ^{14}N medium all the DNA had a density that was exactly intermediate between that of totally ^{15}N DNA and totally ^{14}N DNA. After two generations, half the DNA was of the intermediate density and half was of the density of DNA containing entirely ^{15}N. These observations and those for subsequent generations were exactly what the semi-conservative model predicted.

If the conservative model of DNA replication had been correct, after one generation two bands of DNA would be seen. One band would be in the heavy-density position of the gradient, containing parental DNA molecules, and both strands would consist of ^{15}N-labeled DNA only. The other band would be in the light-density position, containing progeny DNA molecules with both strands totally ^{14}N-labeled. In subsequent generations, the heavy parental DNA band would be seen at each generation, and in the amount found at the start of the experiment. All new DNA molecules would have both strands totally labelled with ^{15}N. Hence, the amount of DNA in the light-density position would increase with each generation. The conservative model of DNA replication, then, the most significant prediction was that at no time would any DNA of

intermediate density have been found. The fact that intermediate-density DNA was found ruled out the conservative model.

In the dispersive model for DNA replication, the parental DNA is scattered in double-stranded segments throughout the progeny DNA molecules. According to this model, all DNA present after one generation in containing medium would be of intermediate density, and this was seen in the Meselson-Stahl experiment. Thus, the dispersive model could not be ruled out after just one generation of replication. After a second generation in ^{14}N-containing medium, the dispersive model predicted that DNA segments from the first generation would be dispersed through the progeny DNA double helices produced. Thus, the ^{15}N-^{15}N DNA segments dispersed among new ^{15}N-^{14}N DNA after one generation would then be distributed among twice as many DNA molecules after two generations. As a result, the DNA molecules would be found at one band located halfway between the intermediate-density and light-density band positions. With subsequent generations, there would continue to be one band and it would become lighter in density with each generation. Such a slow shift in DNA density was not seen in the results of the Meselson-Stahl experiment and therefore the dispersive model was ruled out.

Enzymes Involved in DNA Synthesis

In 1955, Arthur Kornberg and his colleagues set out to find the enzymes that were necessary for the DNA replication so that they could dissect the reactions involved in detail. Kornberg's approach was to identify all the necessary ingredients required for the synthesis of E. coli DNA in vitro. The first successful synthesis of DNA was accomplished in a reaction mixture containing DNA fragments, a mixture of four deoxyribonucleoside 5'-triphosphate precursors (dATP, dGTP, dTTP, and dCTP collectively abbreviated dNTP, for deoxyribo-nucleoside triphosphate), and a lysate prepared from *E. coli* cells. So that he could measure the very small amount of DNA expected to be synthesized in the reaction, Kornberg used radioactively labeled dNTPs in the reaction mixture.

The crucial component (or components) for DNA synthesis must be present in the *E. coli* lysate, Kornberg analyzed the lysate in order to find that component, he isolated an enzyme that was capable of DNA synthesis. This enzyme was originally called the Kornberg enzyme, but it is now most commonly called DNA polymerase-I.

Once DNA polymerase-I was purified, more detailed information could be obtained about DNA synthesis *in vitro*. The first things studied were the various ingredients necessary for the synthesis of DNA *in vitro*. Researchers found that four components were needed for *in vitro* synthesis of DNA. If any one of the following four components were omitted, DNA synthesis would not occur:

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